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NEW PSEUDOPHYLLIDEAN CESTODES FROM THE GREAT LAKES REGION. I. *DIPHYLLOBOTHRIUM OBLONGATUM* N. SP. FROM GULLS*

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Diphyllobothrium oblongatum n. sp. is one of three species of tapeworms belonging to the family DIPHYLLOBOTHRIIDAE which infect young gulls and Caspian terns on Shoe, Hat, and Pismier Islands in northern Lake Michigan. It is described from worms in naturally infected young gulls and from experimentally infected fledgling gulls. These latter birds were fed cysts found on the stomachs of lake herring. The specific name refers to the extremely oblong segments which occur in the posterior third of the strobilae.

Diphyllobothrium oblongatum n. sp.

Specific diagnosis: Small cream-colored worms when alive; total length 28–57 cm; mature segments in posterior third of strobilae longer than broad; 1.75-2 mm broad by 3–7 mm long; greatest thickness 0.419 mm; tandem duplication of genital organs frequent. Largest number of proglottids 455. Scolex, digitate, 1.75 by 0.44 mm; bothria, deep, extend full length, open at the summit; neck short, 1.09 by 0.7 mm, transverse striations extend a short distance before segmentation begins. Genital pore, 44 μ in diameter, median, circular, in papillose area 1.2 by 0.962 mm. Uterine pore, median, circular, papillose, posterior to genital pore by average distance of 166 μ. Uterine loops in loose spiral extend from genital complex anteriorly to cirrus sac with a lateral loop on either side this organ; anterior loops 7–8, walls thick; posterior loops 3–4, narrow, thin-walled. Vitellaria, ovoidal, average 15 by 16 μ in diameter, number approximately 2884, scattered in single layer, in two fields, joined in anterior and posterior portions of each segment. Vitelline ducts, numerous, anastomose to form antler-like connections with oviduct. Testes, ovoidal, average diameter 87 by 131 μ, number 264–318, in single medullary layer, scattered in two fields lateral to ovary, joined in anterior and posterior portions of each segment. Ovary, bilobed, compact; bean-shaped lobes 875–970 μ long by 175–262 μ wide, joined anterior to middle by an isthmus 131–149 by 149–157 μ; lobes meet occasionally at posterior tips. Cirrus, 87 μ at base, when everted 140 μ long. Cirrus sac, pyriform, 175–201 by 306 μ, median, about 1.14–1.0 mm from anterior margin of segment, weakly muscular. Vas deferens extends from apex of ovarian lobes in loose spiral to right and left of median line to join thick-walled, pyriform, seminal vesicle, 70–96 μ in diameter, which is situated posterior to and at base of cirrus sac. Vagina, median, slightly spiral, irregularly alternates to right and left of uterine pore and oviduct; vaginal pore, oval, opens into common genital atrium; seminal receptacle, sleeve-like, 219–330 μ long by 79–96 μ in diameter, thick-walled. Oötype, empty, measures 17–23 μ in diameter. Oöcapt present. Excretory ducts; cortical, numerous, anastomosing; medullary, two main ducts, lateral to uterine coils, continuous proglottid to proglottid. Main nerve trunks two, 44–53 μ wide, lateral to main excretory ducts by width of one to two testes and 394–437 μ from the lateral margins of the proglottid. Mehlis' gland large, diffuse, 219–306 μ wide by 115–306 μ long and 206–306 μ thick, extends ventrally into longitudinal muscles, neither above isthmus nor posteriorly beyond ovarian lobes. Muscles; hypodermal, circular and longitudinal fibers weak; medullary longitudinal, in medium bundles in layer 43–65 μ thick; transverse, few, weak.

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Cortical glands numerous, in layer 43–65 μ thick. Eggs, ovoidal, amber-colored, average measurement 61 by 39 μ, operculum slightly tangential, boss obscure, undeveloped when laid. Coracidia free swimming. Procercoids in *Diaptomus oregonensis*. Plerocercoids in cysts on mesenteries and stomachs of lake herring, *Leucichthys artedi*. Adults in young gulls.

Type host: *Larus argentatus*.
Location: Small intestine.
Type localities: Lakes, Michigan, Huron, and Nipigon.
Type date: July 4, 1943.
Type specimen: U. S. Nat. Mus. Helm. Coll. 36888.

MORPHOLOGICAL RELATIONSHIPS

Talysin (1932) described a tapeworm, *Dibothriocephalus strictus* (= *Diphyllobothrium strictum*), as a parasite from man from the shores of Lake Baikal, Russia. This worm is remarkably similar to *Diphyllobothrium oblongatum* from gulls. His specimen is a small worm of light brownish color, measuring about 47 cm in length. The number of proglottids varies between 180 and 360. The anterior segments are at first shorter than broad. About midway of the entire chain of proglottids the length of a segment is about twice the breadth. The end segments are small in both species. The greatest breadth of a segment in *D. strictum* is 4 mm whereas in *D. oblongatum* the maximum width is 2 mm. In both species, a short distance behind the neck region, faint transverse striations indicate the beginning of proglottid formation. The neck in his specimen is about twice the length of the bothria. In *D. oblongatum* the neck is shorter than the length of the bothria. The scolex is digitate in both species, but the bothria in *D. strictum* do not appear to open to the summit. There is the same tandem duplication of genital organs in both species. Although he does not mention the position of the genital and uterine pores, his figure (Fig. 4) shows them to be in the approximate situations as they occur in *D. oblongatum*. In his specimen (Fig. 6) a clear area is shown anterior to the cirrus sac and posterior to the ovary. In this region apparently the testes and vitellaria are absent. No mention is made of minute papillae which stud the surface in a similar clear area in *D. oblongatum*. The uterus in Talysin's specimen extends from the lobes of the ovary anteriorly in a loose spiral course, but does not have loops on either side of the cirrus sac. No differentiation is made between the anterior and posterior loops of the uterus. No data are given as to the numbers of vitellaria and testes. By scanning his figures, one can see that the ovarian lobes are connected by a short isthmus anterior to their middle. The main longitudinal excretory tubes and lateral nerve trunks are similar to those of the gull tapeworm. The vas deferens alternates to the right and left of the mid line but appears to enter the cirrus sac from its anterior margin without any indication of a seminal vesicle. The diameters of the eggs measure 54–57 by 40 μ. Although there are enough differences between these two tapeworms to set them apart as distinct species, the author believes that data on the thickness of the cortical gland layer and arrangement of muscle layers would give additional information for separating the species of this genus.

Prior to Ariola (1900) many of the reported pseudophyllidean cestodes of birds were placed in the genus *Bothriocephalus*. In some of these descriptions the details are entirely lacking or too inadequate for comparative study. One such worm reported to be in the gulls, *Larus glaucus, L. atricilla, L. canus, L. marinus,* and *L. tridactylus,* is now known as *Tetrabothrium cylindraceum* Rud.

Lühe (1910) contributed greatly towards eliminating this confusion by removing many species from the old genus *Bothriocephalus* and placing the valid ones in the genus *Diphyllobothrium* Cobbold 1858 as parasites of birds and mammals. Lühe redescribed *Diphyllobothrium ditremum* (Crepl.), a tapeworm from North German diving birds, the loons, *Urinator arcticus* (L.), *U. stellatus* (Brunn), and the mergansers, *Mergus merganser* L., and *M. serrator* L. A single specimen was taken from the herring gull, *Larus argentatus* (Brunn). The segments of this worm are 13 mm long by 2.5 mm broad. This length is nearly twice that of a single mature segment of *D. oblongatum.* The scolex is flattened, ovoidal, about 1.2 mm long, 1.0 mm broad, and 0.5 mm thick. No other critical data are supplied. His figure (Fig. 6) of a mature segment does not show lateral loops of the uterus on either side of the cirrus sac.

The above author redescribed another tapeworm, *Diphyllobothrium dendriticum,* which is harbored by North German gulls, *Larus ridibundus* L., *L. canus,* and possibly *L. argentatus,* also the kittiwake, *Rissa tridactyla* (L.). The worm is more robust than *D. oblongatum* as mature segments measure 43 mm in length and 7 mm in breadth. The testes number about 470 and the vitellaria number about 2200. The cirrus sac is about 0.409 mm long and 0.327 mm wide. Since the worm is supposed to resemble *D. ditremum* in other details it is evident that it is a different species than *D. oblongatum.*

Diphyllobothrium exile (= *Dibothrium exile*) was described by Linton (1892) from an immature worm in the California gull, *Larus californicus.* The entire length of the worm is 153 mm. The scolex is spatulate. It measures 1.50 mm long and 0.60 mm at its widest part and is 0.20 mm thick. The neck is long and slender and the bothria do not appear to open to the summit. The details though meager are sufficient to show that it is not *D. oblongatum.*

In 1871 Joseph Leidy named a tapeworm, *Dibothrium cordiceps* (= *Diphyllobothrium cordiceps*), which he found in the white pelican in Yellowstone Park, Wyoming. Since that date no adequate description has been made of the worm. Linton (1891) again found the tapeworm in its normal host, *Pelecanus erythrorhynchus,* from Yellowstone Park. He gives only a few measurements, such as those for the scolex which is 1.00 mm long and 0.75 mm wide and the neck which is 2.00 mm long and 0.60 mm wide. He reaffirms Leidy's (1889) belief that the larval stages are to be found in intestinal cysts and other cysts free in the body cavity of the Rocky Mountain trout, *Salmo mykiss,* Yellowstone Lake, Wyoming. Linton (1892) took immature tapeworms from the California gull, which worms he believed to be *D. cordiceps.* Woodberry (1932) established the relationship between trout cysts and the tapeworms in the white pelican by feeding cysts from the intestines of the trout, *Salmo lewisi,* to young pelicans. He later recovered the adult worms but gave no description of them. Skinker (1932) considered *D. cordiceps* to be identical with *D. latum* as she could not find sufficient characters to separate them. Professor H. B. Ward has made available to the writer specimens of *D. cordiceps,* which in 1926 he collected from the white pelicans in Yellowstone Park. The worms were preserved in corrosive sublimate and stored in 70 per cent alcohol. The shape of the scolex and segments, the arrangement of the ovaries, uterine coils, musculature, and cortical glands, the numbers of the testes and vitellaria all differ from those of *D. oblongatum.* The detailed description of *D. cordiceps* will be forthcoming in a subsequent paper.

The Canadian raven, *Corvus corax principalis,* harbors a tapeworm which was described by Cooper (1921) as *Diphyllobothrium canadense.* The worm is 235 mm long with a maximum width of 6.5 mm. Unlike *D. oblongatum* the posterior segments are 2 to 1½ times as broad as long. Furthermore, the proglottids are "ruffled or frilled laterally." The eggs measure 56–59 μ in length by 37–39 μ in diameter with a large prominent boss. The testes number only about 150.

Vergeer (1942) obtained *Diphyllobothrium laruei* by feeding herring from the Great Lakes to a dog. This species has a total length of 112 mm and is 2.103 mm at its greatest width. "First proglottids very short, gradually increase more rapidly in length than in width until the length of the hindermost gravid segments equals three-fourths their width." The anterior loops of the uterus are thin walled, the middle loops are very thick, and the posterior loops are very thin. The ellipsoidal eggs average 47 μ in length and 33 μ in width. They have an operculum but no boss. The two main medullary excretory trunks are connected by two transverse anastamoses in each segment. Details for this species are mentioned here at this time because Vergeer and the present writer have obtained different species by feeding stomach cysts from herring to different hosts.

Baer (1927) described another one of these small cestodes, *Diphyllobothrium gracile,* from *Felis macura,* Therezopolis, Brazil. It measures 70–80 mm long and 1 mm wide. Midway the segments are quadrate and measure 1 mm by 0.9 mm. The posterior proglottids are 1.83 mm by 1 mm. The ovary is H-shaped. The uterus is only a little coiled and has 4–5 loops to the right and left of the median line. No loops lie lateral or anterior to the cirrus sac. No mention is made of a boss but the eggs are operculate and measure 57 μ by 27 μ.

Observers throughout the world have noted small cestodes of the genus *Diphyllobothrium* which occur in birds and mammals including man. Some have dismissed them as "dwarf" *D. latum* while others have looked for specific differences. Likewise the plerocercoid stage as cysts on the stomachs of herring have been attributed to *D. latum.* Herring generally in the Great Lakes are infected. In order to get a clearer picture of this ecological maze the writer has undertaken for the past ten years a study of life cycles, field observations, and experimental feedings in an attempt to enlarge our knowledge of the true relationships. This part of the study is to be dealt with in subsequent papers.

SUMMARY

1. *Diphyllobothrium oblongatum* n. sp. is described and compared with other cestodes of this same genus which infect gulls, other fish-eating birds, and mammals.

2. This tapeworm was obtained from naturally infected young gulls, and from incubator-reared gulls which were fed stomach cysts from herring, *Leucichthys artedi,* from Lake Michigan and Lake Huron.

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EXPLANATION OF PLATE

All figures were made with the aid of a camera lucida unless otherwise stated. The projected scale to the left of figure one equals 5.0 mm. It was drawn by Baloptican projection from a photograph of the relaxed worm. The scale for figures 2, 3, 4, 5, 6, 9, 10, and 13 equals 0.20 mm and for figures 7, 11, and 12 equals 0.05 mm.

FIG. 1. Mature *Diphyllobothrium oblongatum* from young herring gull experimentally fed plerocercoid stomach-cysts from herring.

FIGS. 2 and 4. Lateral and dorsal views, respectively, of the scolex.

FIGS. 3, 5, and 6. Segments taken at 1.29 mm, 14.19 mm and 35.69 mm, respectively, from the base of the neck.

FIG. 7. Egg from the feces of an experimental herring gull.

FIG. 8. Freehand sketch, dorsal view, of segments near the posterior end of the tapeworm, from a natural infection taken July 3, 1944. Numerous duplications of the genital complex in sequence evident.

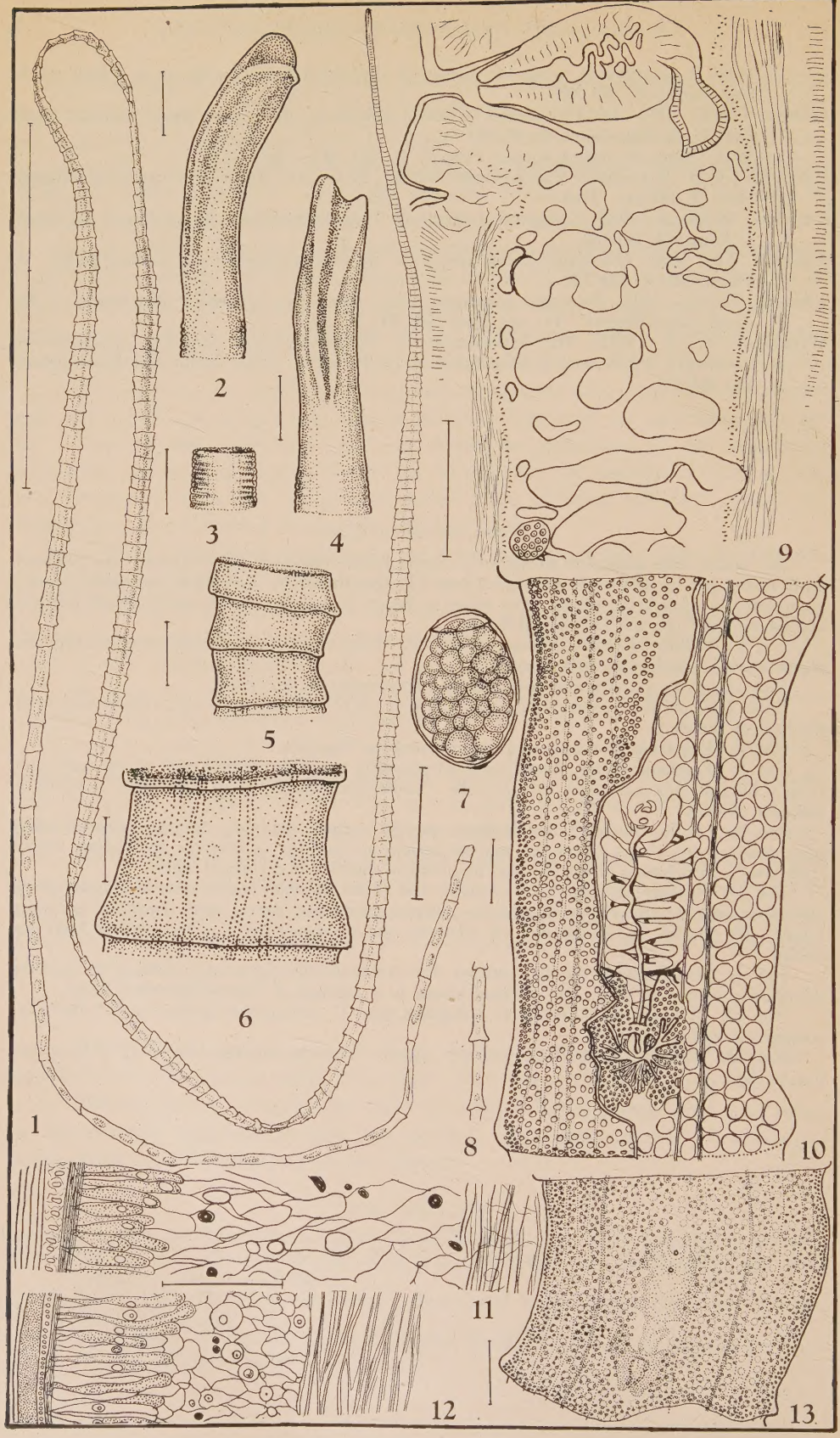
FIG. 9. Outline drawing, longitudinal section, mature segment, showing hermaphroditic pore, uterine pore, cirrus, cirrus sac, seminal vesicle, portions of vas deferens, the vagina, uterine coils, ovary, distribution of circular and longitudinal muscles, and cortical layer.

FIG. 10. Mature segment, near posterior end of the strobila, dorsal view, cut away on the right showing ovarian complex, vagina, vas deferens, cirrus sac, lateral nerve trunk and excretory tubule. On the left the distribution of vitellaria and excretory ducts in the cortical layer are shown.

FIG. 11. Longitudinal section, anterior and dorsal to the cirrus sac, showing in part the distribution of muscle layers, and cortical glands in a segment of *Diphyllobothrium latum*.

FIG. 12. A longitudinal section of a segment from *D. oblongatum* similar to that above for comparison with *D. latum*.

FIG. 13. The one hundred forty-fourth segment from the anterior end of *D. oblongatum*, dorsal view.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THE GENUS *PROTOSTRONGYLUS* KAMENSKIĬ, 1905 (NEMATODA: METASTRONGYLIDAE), AND ITS RELATIVES: PRELIMINARY NOTE[1]

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Several years of work on the nematode lungworms of the *Protostrongylus*-group have resulted in our amassing material and information that will require some time yet for preparation in detailed form appropriate for definitive publication. In the meantime we should like to establish our decisions on the taxonomy of the genera and species known in the group in a preliminary paper in the same manner as one of us (Dougherty, 1943a,b) has already treated certain other lungworms of mammals. For the most part historical data and evidence for our views are left to a later, larger work. However, a fairly extensive list of references is given with this paper to aid anyone in reviewing the literature.

The genus *Protostrongylus* Kamenskiĭ, 1905, and allied genera comprise at least 29 species of which all but three occur in ruminants (order ARTIODACTYLA), the remaining being found in rabbits and hares (order LAGOMORPHA). We regard these genera as constituting a subfamily PROTOSTRONGYLINAE Kamenskiĭ, 1905, of the family METASTRONGYLIDAE Leiper, 1909. Recently one of us (Dougherty, 1945) in a paper on the lungworms of North American deer of the genus *Odocoileus* Rafinesque, 1832, has accepted eight genera in the *Protostrongylus*-group. Since then we have discovered a work by Shul't͡s and Boev (1940) reviewing the lungworms of sheep, both domestic and wild, and proposing another genus—namely *Bicaulus* Shul't͡s and Boev, 1940. However, we regard this to be a synonym of one of the eight—*Varestrongylus* Bhalerao, 1932. In addition to these we add herein two new genera—*Orthostrongylus* gen. n. and *Leptostrongylus* gen. n.—bringing the total of protostrongylin genera to ten.

Orthostrongylus is erected for *Protostrongylus macrotis* Dikmans, 1931, which has already been pointed out (Dougherty, 1945) to possess aberrant features not found in typical species of *Protostrongylus*. *Leptostrongylus* is erected for two species of lungworms in deer with *Pneumostrongylus alpenae* Dikmans, 1935, as genotype. We now feel that an error was made (by Dougherty, 1945) in lumping these species in the same genus with *Varestrongylus pneumonicus* Bhalerao, 1932, type of *Varestrongylus*.

Almost alone the Soviet helminthologists persist in the use of the generic name *Synthetocaulus* Raillliet and Henry, 1907, in place of the earlier name *Protostrongylus*, of which the former is a subjective synonym. We have already expressed our opinion on this question (Goble and Dougherty, 1943), but may repeat here

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[1] Our appreciation is expressed to Dr. Gerard Dikmans, Zoölogical Division, United States Bureau of Animal Industry, Beltsville, Maryland, for advice and criticism.

the fact that the genotype of *Synthetocaulus,* namely *Strongylus commutatus* Diesing, 1851 (= *Filaria pulmonalis* v. Frölich, 1802), as selected by Railliet and Henry (1907), and the genotype of *Protostrongylus,* namely *Strongylus rufescens* Leuckart, 1865, as selected by Cameron (1927), are clearly congeneric, and therefore the former generic name must by the Rules of Zoölogical Nomenclature fall as a synonym of the latter.

Following is a key to the ten genera of the PROTOSTRONGYLINAE. After the key they are listed with their synonyms, genotypes, and species and with diagnoses for *Orthostrongylus* and *Leptostrongylus.* Where generic or trivial names are here reduced to synonymy for the first time, such are marked with an asterisk (*). The list of references at the end includes the works in which first appeared the generic and trivial names here listed, whether valid or synonymous. Works in which new combinations were formed or in which names arising as *lapsus calami* appeared are not listed unless specifically indicated or mentioned elsewhere in this text. In a few cases there are additional notes on nomenclatorial matters which are not covered in the literature.

1. Bursa strongly reduced with some bursal rays lost ..... *Muellerius*
   Bursa more or less normally developed 2
2. Crura of gubernaculum relatively stocky and considerably shorter than corpus; capitulum absent; arcus present or absent 3
   Crura of gubernaculum slender and longer or little shorter than corpus; capitulum present; arcus present 7
3. Arcus elaborately developed; spicules very unequal ..... *Neostrongylus*
   Arcus absent; spicules equal 4
4. Corpus split distally for about half its length ..... *Leptostrongylus* gen. n.
   Corpus not split distally 5
5. Corpus projecting between crura some distance posteriorly and coming to a point ..... *Varestrongylus*
   Corpus not projecting posteriorly beyond crura 6
6. Dorsal ray completely divided into two stalks ..... *Elaphostrongylus*
   Dorsal ray mound-like, not divided into two stalks ..... *Pneumostrongylus*
7. Arcus very elaborately developed, capitulum reduced ... *Orthostrongylus* gen. n.
   Arcus simple, capitulum well developed 8
8. Dorsal ray slender with long terminal papillae ..... *Cystocaulus*
   Dorsal ray mound-like with scattered papillae 9
9. Spicules long (over 0.9 mm.) and capillary ..... *Spiculocaulus*
   Spicules relatively short (under 0.6 mm.) and stout ..... *Protostrongylus*

Phylum NEMATODA

Class PHASMIDEA

Order RHABDITIDA—Suborder STRONGYLINA

Family METASTRONGYLIDAE

Subfamily PROTOSTRONGYLINAE

Protostrongylus Kamenskiĭ, 1905

Synonymy.—*Strongylus* Müller, 1780 (*partim*); *Synthetocaulus* Railliet and Henry, 1907.

Genotype.—*P. rufescens* (Leuckart, 1865) Kamenskiĭ, 1905.

Species.—

Protostrongylus rufescens (Leuckart, 1865) Kamenskiĭ, 1905 (see also: Koch, 1883; Lutz, 1926; Shul'ts and Boev, 1940).

Synonymy.—*Strongylus rufescens* Leuckart, 1865; *Synthetocaulus rufescens* (Leuckart, 1865) Railliet and Henry, 1907; *Sclerostoma rufescens* (Leuckart, 1865) Braun and Lühe, 1910; *Metastrongylus rufescens* (Leuckart, 1865) Sluiter and Swellengrebel, 1912; *Strongylus* (*Synthetocaulus*) *rufescens* Leuckart, 1865, of Hutyra and Marek, 1913; *Dictyocaulus rufescens* (Leuckart, 1865) Pratt, 1916; *Strongylus* (*Synthetocaulus*) *rufescens ovis* Leuckart-Koch, of Lutz, 1926; *Synthetocaulus kochi* Shul'ts, Orlov, and Kutas, 1933; *Protostrongylus kochi* (Shul'ts, Orlov, and Kutas, 1933) Chitwood and Chitwood, 1938.

Type Host.—Domestic sheep, *Ovis aries* Linné.

Protostrongylus unciphorus (Railliet and Henry, 1907) Cameron, 1927 (see also: Shul'ts, Orlov, and Kutas, 1933; Boev, 1936; Mikačić, 1940; Shul'ts and Boev, 1940).

Synonymy.—*Synthetocaulus unciphorus* Railliet and Henry, 1907; **Synthetocaulus hobmaieri* Shul'ts, Orlov, and Kutas, 1933; *Protostrongylus hobmaieri* (Shul'ts, Orlov, and Kutas, 1933) Cameron, 1934.

Syntype Hosts.—Domestic sheep, *Ovis aries* Linné; and Domestic goat, *Capra hircus* Linné.

Protostrongylus cameroni (Shul'ts and Boev, 1940) comb. n. (see also: Cameron, 1927; Baylis, 1929).

Synonymy.—*Protostronglus ocreatus,* of Cameron, 1927 (*nec Synthetocaulus ocreatus* Railliet and Henry, 1907); *Synthetocaulus cameroni* Shul'ts and Boev, 1940.

Type Host.—Domestic sheep, *Ovis aries* Linné.

Protostrongylus skrjabini (Boev, 1936) Dikmans, 1945 (see also: Shul'ts and Boev, 1940; Boev, 1940; Dikmans, 1945a).

Synonymy.—*Synthetocaulus skrjabini* Boev, 1936; *Protostrongylus gracilis* Dikmans, 1945.

Type Host.—Domestic sheep, *Ovis aries* Linné.

Protostrongylus brevispiculum Mikačić, 1940.

Synonymy.—*Protostrongylus rupicaprae brevispiculum* Mikačić, 1940.

Type Host.—Domestic sheep, *Ovis aries* Linné.

Protostrongylus stilesi Dikmans, 1931; (see also: Shul'ts, Orlov, and Kutas, 1933; Boev, 1936; Mikačić, 1939; Shul'ts and Boev, 1940).

Synonymy.—*Synthetocaulus stilesi* (Dikmans, 1931) Shul'ts, Orlov, and Kutas, 1933; **Synthetocaulus raillieti* Shul'ts, Orlov, and Kutas, 1933; *Protostrongylus raillieti* (Shul'ts, Orlov, and Kutas, 1933) Cameron, 1934; ? **Protostrongylus Frosti* (*sic*) Honess, 1942.

Type Host.—Common Rocky Mountain sheep, *Ovis c. canadensis* Shaw (also found in domestic sheep in Kazakhstan, U.S.S.R.).

Note.—Dr. G. Dikmans reports (*in litt.*) that specimens referred to the Zoölogical Division of the U. S. Bureau of Animal Industry from Honess's study were identified as *P. rushi* and *P. stilesi* although later reported as *P. rushi* and *P. frosti* by Honess (1942). However, one male specimen in the material at the Bureau appears to be a distinct species, but is in a permanent mount that precludes complete study. Although in Honess's description of *P. frosti* there is nothing to distinguish it clearly from *P. stilesi,* we place it only tentatively in synonymy with the latter. It seems probable to us that *P. frosti* is not distinct from *P. stilesi* and that the odd male belongs to another species not described or reported by Honess.

Protostrongylus rushi Dikmans, 1937 (see also: Dikmans, 1943).

Synonymy.—*Synthetocaulus rushi* (Dikmans, 1937) Shul'ts and Boev, 1940.

Type Host.—Common Rocky Mountain sheep, *Ovis c. canadensis* Shaw.

Protostrongylus rupicaprae Gebauer, 1932 (see also: Lutz, 1926).

Synonymy.—*Strongylus* (*Synthetocaulus*) *rufescens rupicaprae* I Stroh, of Lutz, 1926; *Synthetocaulus rupicaprae* (Gebauer, 1932) Shul'ts, Orlov, and Kutas, 1933.

Type Host.—Chamois, *Rupicapra rupicapra* (Linné).

Protostrongylus coburni Dikmans, 1935.

Type Host.—Northern white-tailed deer, *Odocoileus virginianus borealis* Miller.

Protostrongylus pulmonalis (v. Frölich, 1802) Goble and Dougherty, 1943 (see also: Lutz, 1926; Joyeux, Sautet, and Cabassu, 1939; Scott, 1943; Goble and Cheatum, 1944).

Synonymy.—*Filaria pulmonalis* v. Frölich, 1802; *Filaria leporis pulmonalis* Rudolphi, 1819; *Strongylus retortaeformis* var. *major* Rudolphi, 1819; *Strongylus commutatus* Diesing, 1851; *Trichosoma leporis* Diesing, 1851; *Trichosoma leporis pulmonale* (Rudolphi, 1819) Diesing, 1851; *Filaria terminalis* Passerini, 1884; *Filaria leporis pulmonalis* v. Frölich, of Kamenskiĭ, 1905; *Protostrongylus terminalis* (Passerini, 1884) Kamenskiĭ, 1905; *Protostrongylus leporis* Kamenskiĭ 1905; "Large form" of Nikulin in Kamenskiĭ, 1905; *Synthetocaulus commutatus* (Diesing, 1851) Railliet and Henry, 1907; *Capillaria leporis* (Diesing, 1851) Travassos, 1915; *Synthetocaulus pulmonalis* (v. Frölich, 1802) Hall, 1916; *Capillaria pulmonalis* (Froel., 1802) Rud., 1819, of Hall, 1916; *Strongylus* (*Synthetocaulus*) *rufescens leporis* Diesing-A. Mueller, of Lutz, 1926; *Synthetocaulus terminalis* (Passerini, 1884) Shul'ts, Orlov, and Kutas, 1933; *Proto-*

strongylus commutatus (Diesing, 1851) Kamenskiĭ, 1905, of Cameron, 1927; **Protostrongylus sylvilagii* Scott, 1943; *Protostrongylus sylvilagi* Scott, 1943, emend. Goble and Cheatum, 1944.

Type Host.—Common European hare, *Lepus e. europaeus* Pallas.

Protostrongylus kamenskyi Shul'ts, 1930.

Synonymy.—"Small form" of Nikulin, in Kamenskiĭ, 1905; *Synthetocaulus kamenskyi* (Shul'ts, 1930) Shul'ts, Orlov, and Kutas, 1933.

Type Host.—Kozhevnikov varying hare, *Lepus timidus kozhevnikovi* Ognev.

Protostrongylus boughtoni Goble and Dougherty, 1943 (see also; Boughton, 1932).

Synonymy.—*Synthetocaulus leporis* Boughton, 1932; *Synthelocaulus* [*sic*] *cuniculi* Green and Shillinger, 1935 (*nom. nud.*); *Protostrongylus leporis* (Boughton, 1932) MacLulich, 1937 (*nec Protostrongylus leporis* Kamenskiĭ, 1905).

Type Host.—Common American varying hare, *Lepus a. americanus* Erxleben.

Spiculocaulus Shul'ts, Orlov, and Kutas, 1933.

Synonymy.—*Protostrongylus* Kamenskiĭ, 1905 (partim); *Spiculostrongylus* Cameron, 1934 (*err. pro. Spiculocaulus*).

Genotype.—*S. leuckarti* Shul'ts, Orlov, and Kutas, 1933.

Species.—

Spiculocaulus leuckarti Shul'ts, Orlov, and Kutas, 1933 (see also: Shul'ts and Boev, 1940).

Type Host.—Domestic sheep, *Ovis aries* Linné.

Spiculocaulus kwongi (Wu and Liu, 1943) comb. n. (see also: Dikmans, 1945b).

Synonymy.—*Protostrongylus kwongi* Wu and Liu, 1943.

Syntype Hosts.—Domestic sheep, *Ovis aries* Linné; and Domestic goat, *Capra hircus* Linné.

Spiculocaulus austriacus (Gebauer, 1932) comb. n.

Synonymy.—*Protostrongylus austriacus* Gebauer, 1932: *Synthetocaulus austriacus* (Gebauer, 1932) Shul'ts and Boev, 1940.

Type Host.—Chamois, *Rupicapra rupicapra* (Linné).

Cystocaulus Shul'ts, Orlov, and Kutas, 1933.

Synonymy.—*Strongylus* Müller, 1780 (*partim*); *Synthetocaulus* Railliet and Henry, 1907 (*partim*); *Protostrongylus* Kamenskiĭ, 1905 (*partim*).

Genotype.—*C. ocreatus* (Railliet and Henry, 1907) Mikačić, 1939.

Species.—

Cystocaulus ocreatus (Railliet and Henry, 1907) Mikačić, 1939 (see also: Lutz, 1926; Shul'ts and Boev, 1940).

Synonymy.—*Synthetocaulus ocreatus* Railliet and Henry, 1907; *Strongylus nigrescens* Jerke, 1911; *Strongylus* in den subpleuralen Knoten, of Hueber, 1925; *Strongylus* Utz-Lydtin,

of Lutz, 1926; *Strongylus* (*Synthetocaulus*) *nigrescens ovis* Utz-Jerke, of Lutz, 1926; *Protostrongylus ocreatus* (Railliet and Henry, 1907) Cameron, 1927 (*nec Protostrongylus ocreatus*, of Cameron, 1927); *Protostrongylus nigrescens* (Jerke, 1911) Gebauer, 1932; *Cystocaulus nigrescens* (Jerke, 1911) Shul'ts, Orlov, and Kutas, 1933.

Type Host.—Domestic sheep, *Ovis aries* Linné.

ORTHOSTRONGYLUS[2] GEN. N.

Synonymy.—*Protostrongylus* Kamenskiĭ, 1905 (*partim*).

Diagnosis.—Prostrongylinae: body filiform; cuticle smooth; stoma vestigial. Male: bursa bilobed; dorsal ray thickened, rounded, and provided with pedunculate papillae; arcus elaborately developed; spicules with two pectinate alae each and with simple terminations; telamon not apparent; gubernaculum consisting of atypical capitulum without the usual lateral projections, reduced corpus doubled back from the crura, and well developed, long, thin crura provided at their ends with weakly sclerotized pedes. Female: provagina absent; genitalia characteristic.

Genotype.—*O. macrotis* (Dikmans, 1931) comb. n.

Species.—

Orthostrongylus macrotis (Dikmans, 1931) comb. n. (see also: Dougherty, 1945).

Synonymy.—*Protostrongylus macrotis* Dikmans, 1931; *Synthetocaulus macrotis* (Dikmans, 1931) Shul'ts, Orlov, and Kutas, 1933.

Type Host.—Rocky Mountain mule deer, *Odocoileus h. hemionus* (Rafinesque).

Neostrongylus Gebauer, 1932

Synonymy.—*Synthetocaulus* Railliet and Henry, 1907 (*partim*); *Protostrongylus* Kamenskiĭ, 1905 (*partim*); *Neostrongylus* Gebauer, 1931 (*nom. nud.*)

Genotype.—*N. linearis* (Marotel, 1913) Gebauer, 1932.

Species.—

Neostrongylus linearis (Marotel, 1913) Gebauer, 1932.

Synonymy.—*Synthetocaulus linearis* Marotel, 1913; *Protostrongylus linearis* (Marotel, 1913) Baylis, 1929.

Type Host.—Domestic sheep, *Ovis aries* Linné.

LEPTOSTRONGYLUS[3] GEN. N.

Synonomy.—*Pneumostrongylus* Mönnig, 1932 (*partim*); *Varestrongylus* Bhalerao, 1932 (*partim*).

Diagnosis.—Protostrongylinae: body filiform, cuticle smooth; stoma vestigial. Male: bursa undivided; dorsal ray relatively slender (?in *L. capreoli*) and provided with sessile papillae; arcus absent; spicules with two pectinate alae each and with simple terminations; telamon well developed; gubernaculum consisting of weakly sclerotized corpus split in its posterior half and well sclerotized, short, stocky crura. Female: provagina present; genitalia characteristic.

Genotype.—*L. alpenae* (Dikmans, 1935) comb. n.

Species.—

Leptostrongylus alpenae (Dikmans, 1935) comb. n. (see also: Dougherty, 1945).

Synonymy.—*Pneumostrongylus alpenae* Dikmans, 1935; *Varestrongylus alpenae* (Dikmans, 1935) Dougherty, 1945.

Type Host.—Northern white-tailed deer, *Odocoileus virginianus borealis* Miller.

Leptostrongylus capreoli (Stroh and Schmid, 1938) comb. n.

Synonymy.—*Protostrongylus capreoli* Stroh and Schmid, 1938; *Varestrongylus capreoli* (Stroh and Schmid, 1938) Dougherty, 1945.

Type Host.—Common roe deer, *Capreolus c. capreolus* (Linné).

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[2] From ὀρθός, in line (with); στρογγύλος, round.

[3] From λεπτός, thin; στρογγύλος, round.

Pneumostrongylus Mönnig, 1932.

Genotype.—*P. calcaratus* Mönnig, 1932.

Species.—

Pneumostrongylus calcaratus Mönnig, 1932 (see also: Mönnig, 1933).

Type Host.—Common impalla, *Aepyceros m. melampus* (Lichtenstein).

Pneumostrongylus tenuis Dougherty, 1945.

Type Host.—Common white-tailed deer, *Odocoileus v. virginianus* (Boddaert).

Elaphostrongylus Cameron, 1931.

Genotype.—*E. cervi* Cameron, 1931.

Species.—

Elaphostrongylus cervi Cameron, 1931.

Type Host.—Scottish red deer, *Cervus elephas scoticus* Lönnberg.

Elaphostrongylus odocoilei Hobmaier and Hobmaier, 1934.

Type Host.—Columbian black-tailed deer, *Odocoileus hemionus columbianus* (Richardson).

Varestrongylus Bhalerao, 1932.

Synonymy.—*Strongylus* Müller, 1780 (*partim*); *Protostrongylus* Kamenskiĭ, 1905 (*partim*); *Synthetocaulus* Railliet and Henry, 1907 (*partim*); **Bicaulus* Shul'tŝ and Boev, 1940.

Genotype.—*V. pneumonicus* Bhalerao, 1932.

Species.—

Varestrongylus pneumonicus Bhalerao, 1932 (see also: Dougherty, 1945).

Syntype Hosts.—Bharal, or wild blue sheep, *Pseudoïs nayaur* (Hodgson); Hodgson argali, *Ovis ammon hodgsoni* Blyth; Filippi Siberian ibex, *Capra sibirica filippii* Camerano; "Tibetan goat," *C. sibirica* subsp.?

Varestrongylus schulzi (Boev and Vol'f, 1938) comb. n. (see also: Shul'tŝ and Boev, 1940; Dikmans, 1945a).

Synonymy.—*Synthetocaulus schulzi* Boev and Vol'f, 1938; *Bicaulus schulzi* (Boev and Vol'f, 1938) Shul'tŝ and Boev, 1940; *Varestrongylus sinicus* Dikmans, 1945.

Type Host.—Domestic sheep, *Ovis aries* Linné.

Varestrongylus sagittatus (Mueller, 1890) Dougherty, 1945 (see also: Schmid, 1939).

Synonymy.—*Strongylus sagittatus* Mueller, 1890; *Synthetocaulus sagittatus* (Mueller, 1890) Railliet and Henry, 1907; *Protostrongylus sagittatus* (Mueller, 1890) Cameron, 1927.

Type Host.—German red deer, *Cervus elephas germanicus* Desmarest.

Note.—This species was recently placed in *Varestrongylus* (by Dougherty, 1945) because the corpus of its gubernaculum as figured by Mueller (1890, Abb. 4) agrees with that of *V. pneumonicus* as defined for *Varestrongylus* in our key. However, Mueller's figure may have been a reconstruction, and, judging by a photomicrograph of *V. sagittatus* appear-

ing in the work of Schmid (1939, Abb. 3), we feel that Mueller possibly inverted the corpus of the gubernaculum in his figure. If so, *V. sagittatus* fits nicely into *Leptostrongylus.* However, Schmid's illustration is only suggestive, and we have no choice at this time other than to leave Mueller's species in *Varestrongylus* pending further study.

Muellerius Cameron, 1927.

Synonymy.—*Strongylus* Müller, 1780 (*partim*); *Pseudalius* Dujardin, 1844 (*partim*); *Synthetocaulus* Railliet and Henry, 1907 (*partim*).

Genotype.—*M. minutissimus* (Mégnin, 1878) comb. n.

Species.—

Muellerius minutissimus (Mégnin, 1878) comb. n. (see also: Koch, 1883; Schlegel, 1899; Cameron, 1927; Gebauer, 1932).

Synonymy.—*Nematoideum ovis pulmonale* Diesing, 1851; *Strongylus minutissimus* Mégnin, 1878; *Pseudalius ovis pulmonalis* (Diesing, 1851) Koch, 1883; *Pseudalius capillaris* Mueller, 1889; *Strongylus ovis pulmonalis* (Diesing, 1851) Curtice, 1890; *Pseudalius ovis* Brown, 1897; *Strongylus ovis pulmonaris,* of Gillruth, 1903; *Synthetocaulus capillaris* (Mueller, 1889) Railliet and Henry, 1907; *Muellerius capillaris* (Mueller, 1889) Cameron, 1927.

Type Host.—Domestic sheep, *Ovis aries* Linné.

Muellerius tenuispiculatus Gebauer, 1932.

Type Host.—Chamois, *Rupicapra rupicapra* (Linné).

SUMMARY

The subfamily PROTOSTRONGYLINAE Kamenskiĭ, 1905, of the strongyline family METASTRONGYLIDAE Leiper, 1909, is herein briefly reviewed. A key to the ten accepted genera and a list of these genera and their 29 species with synonymies are given. With each species is listed its type host or syntype hosts. Two of the ten genera are erected as new—*Orthostrongylus* gen. n. with genotype *O. macrotis* (Dikmans, 1931) comb. n. (syn. *Protostrongylus macrotis*) and *Leptostrongylus* gen. n. with genotype *L. alpenae* (Dikmans, 1935) comb. n. (syn. *Pneumostrongylus,* or *Varestrongylus alpenae*).

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[7] Apparently not available in North America; reference in Stiles and Hassall (1907, U. S. Bur. Animal Ind. Bull. No. 39, p. 1479).

ALARIA TAXIDEAE N. SP., FROM THE BADGER AND OTHER MUSTELIDS[1]

GUSTAV SWANSON[2] AND ARNOLD B. ERICKSON

Only two species of the fluke genus *Alaria, mustelae* and *freundi,* have been described from North American mustelid mammals. A number of years ago one of us (G.S.) tentatively described a new *Alaria,* based on four specimens, from the badger (*Taxidea taxus*). More recently we have recovered additional specimens of this fluke from the badger and other mustelids in Minnesota. Only the adult flukes, however, have been found; nothing is known of the immature stages.

The following description of the species is based on a total of 37 specimens. Both contracted and relaxed specimens were studied, and sagittal, longitudinal, and transverse sections were made. In the description measurements of length precede those of width.

Alaria (*Paralaria*) *taxideae* n. sp.

Description: Total length 1.10–2.27 mm (av. 1.82 mm). Forebody 0.64–1.46 mm by 0.62–1.21 mm (av. 1.04 by 0.90 mm); hindbody 0.46–1.37 mm by 0.45–0.97 mm (av. 0.82 by 0.72 mm). Holdfast organ 0.38–0.97 mm by 0.29–0.84 mm (av. 0.66 by 0.51 mm); may or may not cover the acetabulum. Oral sucker 78–178 μ by 78–152 μ (av. 115.6 by 123.8 μ). Esophagus very short or lacking. The intestine is large, extending back to the posterior boundary of the last testis. It is ventro-lateral in position in the hindbody. Lateral sucking cups on each side of the oral sucker, may be inverted or everted. When everted they project about 100 μ beyond the contour of the body. Pharynx much larger than the oral sucker 122–240 μ by 95–190 μ (av. 172 by 147.5 μ). Acetabulum 65–106 μ by 57–114 μ (av. 87 by 80 μ). Vitellaria distributed principally through the forebody and to a limited extent in the hindbody to the anterior testis. Ovary, 102.6–140.6 μ long by 152–298 μ wide (av. 125.4 by 216 μ), is situated dorsally just in front of the testis near the junction of the two body regions. The ascending limb of the uterus enters the posterior end of the holdfast organ. The descending limb passes posteriad along the ventral side to the genital cone where it is joined by the ejaculatory duct just before their common opening into the genital atrium. Eggs 25 to 55 in a well-filled uterus, but some specimens contain only five or six. Eggs 106–143 μ by 60–82 μ (av. 114.4 by 65.5 μ). The vitelline reservoir lies between the two testes on the left side; Mehlis' gland is dorsal and lies lateral to or postero-lateral to (left of) the anterior testis. When in the postero-lateral position, it occupies the opening between the anterior and posterior testis. Anterior testis 193.8–240 μ long by 247–447 μ wide (av. 217 by 341.3 μ), divided into two lobes by a slight cleft; left lobe smaller than the right lobe, sometimes much attenuated. Posterior testis 212.8–308 μ long by 285–744 μ wide (av. 253.4 by 457 μ). It is roughly horseshoe-shaped and three-lobed, the tips extending laterally and posteriorly so as to include part of the seminal vesicle. The vas deferens descends on the ventral side of the testes just dorsal to, or beside, the uterus. The ejaculatory duct originates dorsally just behind the second testis and extends posteriad and ventrally to the genital cone. The genital pore is subterminal and dorsal. The coiled seminal vesicle occupies considerable space behind the second testis.

Relationships: Alaria taxideae, thus named because it was first recovered from the badger (*Taxidea taxus*), is characterized by the fact that its pharynx, in comparison with the oral and ventral suckers, is very large. In body size it is similar to but somewhat larger than *Alaria mustelae, A. freundi,* and *A. intermedia,* and its pharynx is much larger than the pharynx of any one of these species. Also its posterior testis is three-lobed and horseshoe-shaped; the posterior testes of the above three species are not horseshoe-shaped. The shape of the anterior testis of *A. taxideae* is noticeably larger at the right end than at the left end, and the seminal vesicle is unusually broad. Its pharynx is more nearly the size of the pharynx of *A. clathrata* but is larger despite the fact that the body length of *A. clathrata* is more than twice that of *A. taxideae.* The

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posterior testis of *A. nasuae* is horseshoe-shaped as is that of *A. taxideae*. Although *A. nasuae* is much larger than *A. taxideae*, it has a much smaller pharynx than the latter.

Hosts: Badger (*Taxidea taxus*), short-tailed weasel (*Mustela cicognanii*), long-tailed weasel (*Mustela frenata*), striped skunk (*Mephitis mephitis*), and spotted skunk (*Spilogale putorius*).

Location: Small intestine.

Distribution: Minnesota.

Type specimens: Holotype, (from a badger collected 12–14–29), University of Minnesota Collection, Tr. 83 S1. 23. Paratypes also in the University of Minnesota Collection, St. Paul, Minn.

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Explanation of Plate

Fig. 1. Whole mount of contracted specimen, ventral aspect.

Fig. 2. Partial reconstruction of sagittal sections.

Fig. 3. Transverse section through forebody at level of acetabulum.

Fig. 4. Transverse section through forebody at middle of holdfast.

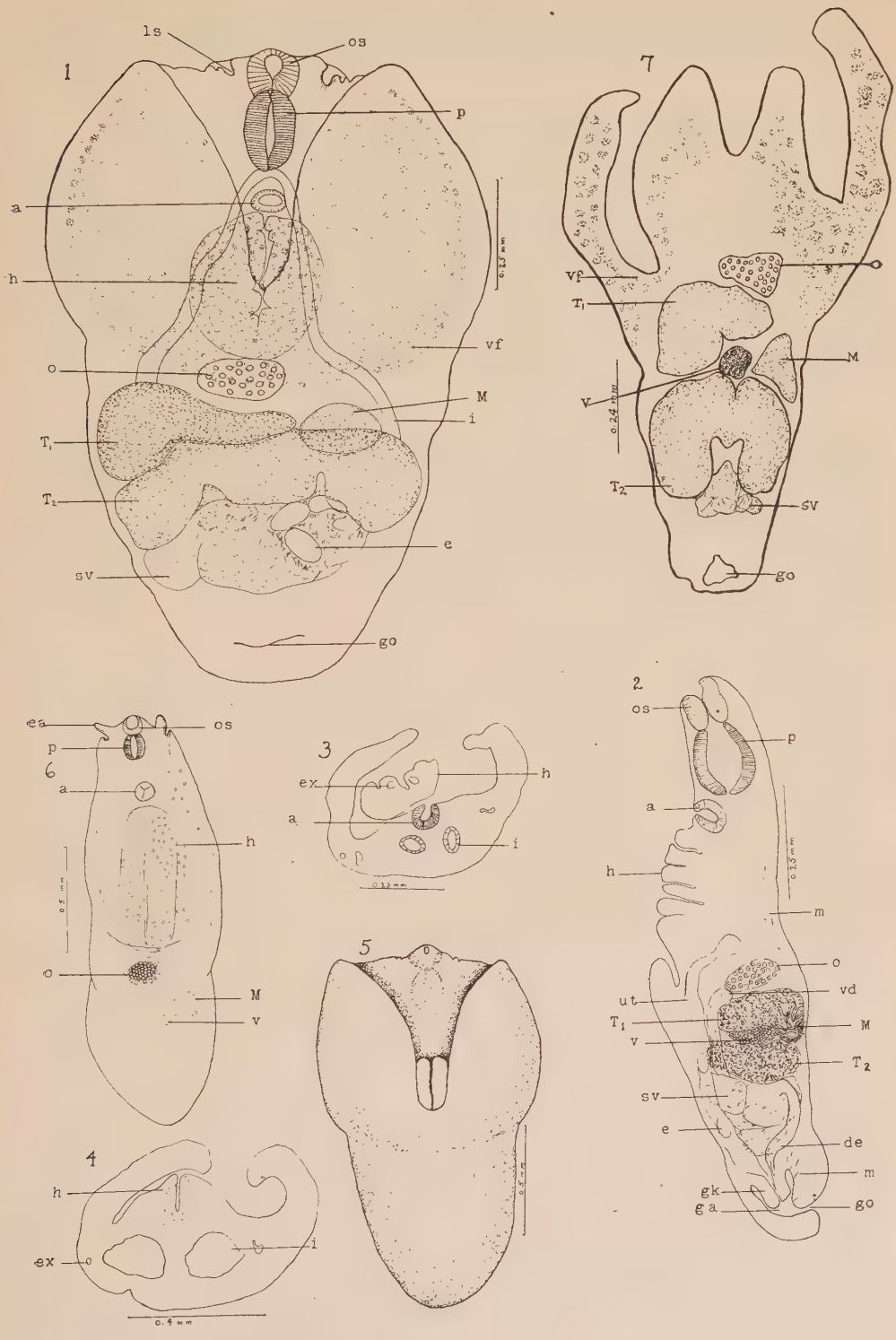
Fig. 5. Uncleared specimen.

Fig. 6. Whole mount of sexually degenerating specimen, flattened, and with ear-like appendages protruded.

Fig. 7. Longitudinal section of a partially relaxed specimen.

Abbreviations

a	acetabulum	m	Mehlis' gland
de	ejaculatory duct	o	ovary
e	egg	os	oral sucker
ea	ear-like appendage	p	pharynx
ex	excretory space	sv	seminal vesicle
ga	genital atrium	T1	anterior testis
gk	genital cone	T2	posterior testis
go	genital opening	ut	uterus
h	holdfast	v	vitelline reservoir
i	intestine	vd	vas deferens
ls	lateral sucking cup	vf	vitelline follicles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THE EFFECT OF SOME ARYL ARSONIC ACIDS ON EXPERIMENTAL COCCIDIOSIS INFECTION IN CHICKENS

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Numerous preparations have been suggested during the last few years for the prevention and treatment of bloody cecal coccidiosis in chickens. It appears unquestionable that sulfur and some compounds of the sulfa group are able to modify a coccidial infection, although no completely satisfactory compound has yet been reported. In fact, some of the preparations are known to produce pathological conditions which make them undesirable. Only a few attempts to use arsenical preparations for the control of coccidiosis have been reported in the literature and none of them were reported as effective.

Investigation concerning the value of arsenic compounds in the control of coccidiosis has been in progress at this laboratory for several years. Two aryl arsonic acids have given better results than any arsenicals previously tested, namely, 4-hydroxy phenylarsonic acid and 3-nitro 4-hydroxy phenylarsonic acid and their sodium salts.

The 4-hydroxy phenylarsonic acid used in these experiments was obtained either from the Mallinckrodt Chemical Co. or it was produced by the chemical department of Dr. Salsbury's Laboratories. The 3-nitro 4-hydroxy phenylarsonic acid was produced in our laboratory.

The chicks used in these experiments were battery-raised New Hampshire chicks from 3.5 weeks to 9 weeks of age. During the brooding period the usual precautions were taken to insure the freedom of these birds from all coccidiosis infection. The experiments were conducted in individual wire-floored cages, in wire-floored brooding batteries or in 14′ × 16′ brooder houses.

An estimate of the comparative number of oöcysts eliminated by treated and untreated chicks was used as a criterion for the efficacy of the arsenical preparation against the intestinal coccidium *Eimeria acervulina*. This estimate was obtained by examining the droppings of each chick from the 4th to 8th days of the patent period by the simple salt-flotation technique using saturated NaCl as the flotation medium. The efficacy of these preparations against the cecal coccidium *Eimeria tenella* was judged by the amount of blood passed in the droppings during the patent period and by the mortality in treated and untreated groups. Weight records, water-consumption records and feed-consumption records were used as an indication of the degree of toxicity.

In the first experiment, eight 7-week-old New Hampshire chicks were placed in individual cages. Four of these chicks received no medication, while four of them received 4-hydroxy phenylarsonic acid in their drinking water at concentrations of 0.1, 0.2, 0.3 and 0.4%, respectively. These chicks were infected with approximately 150,000 sporulated oöcysts of *E. acervulina* three days after medication was begun and received approximately 100,000 sporulated oöcysts of *E. tenella* seven days following the beginning of medication.

The chicks receiving concentrations of 0.3 and 0.4% of this compound died showing typical lesions of arsenic poisoning on the 10th and 2nd days of medication,

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respectively. Although water-consumption records and weight records of the chicks receiving the lower dosages indicated considerable toxicity, both birds survived the treatment.

No coccidia oöcysts could be found in the droppings of the medicated chicks on the 4th to 8th days following infection with *E. acervulina,* whereas all four control chicks produced an abundance of oöcysts during this same period.

Neither of the two chicks surviving the medication in the treated group passed any blood in the droppings following their infection with *E. tenella,* whereas the controls all showed moderate to severe hemorrhage during the patent period, all hemorrhage having ceased without death loss by the 9th day after infection.

Since no clinical symptoms or other evidence of infection were detected in the treated birds by the laboratory methods used in the previous experiment, it was desirable to learn whether lower doses of 4-hydroxy phenylarsonic acid, relatively non-toxic to chickens, might also produce similar results.

Eight Barred Rock chicks 8 weeks old were placed in individual cages. Two of these received 0.05% of 4-hydroxy phenylarsonic acid in their drinking water, two received this compound at a concentration of 0.025%, and four unmedicated chicks were held as controls. Each chick received approximately 150,000 sporulated oöcysts of *E. acervulina* two days after medication was begun and approximately 135,000 sporulated oöcysts of *E. tenella* four days later. Water consumption in the groups receiving 0.05 and 0.025% of the arsonic acid in their drinking water was respectively 88.8 and 105.1% of the controls while their weights were respectively 100.4 and 108.8% of the controls.

This arsonic acid was of no value at concentrations of 0.025 and 0.05% in the drinking water for the prevention of *E. acervulina* infection since oöcysts were approximately as numerous in the droppings of the treated birds as in the controls. The treated chicks passed no blood in their droppings following their infection with large numbers of sporulated oöcysts of *E. tenella,* whereas all the controls suffered severe hemorrhage.

While chicks receiving 4-hydroxy phenylarsonic acid at doses which were relatively non-toxic failed to show clinical symptoms of cecal coccidiosis infection, it was desirable to test the effect of the sodium salt of this compound since it is more readily prepared. Preliminary experiments showed that sodium 4-hydroxy phenylarsonate was effective against *E. tenella* in comparatively non-toxic doses and that it was likewise effective when combined in a tablet containing sodium phenolsulphonate, ammonium phenolsulphonate, lactose and boric acid.

Twenty-four New Hampshire chicks 7 weeks old were placed in individual cages and divided into two groups of 12 chicks each. One group received a phenolsulphonate-lactose-boric acid preparation containing sodium 4-hydroxy phenylarsonate at a dosage which gave a concentration of 0.027% of the arsenic compound in the drinking water. The other group received no medication and thus served as a control for the experiment. Each of the 24 chicks received approximately 100,000 sporulated oöcysts of *E. tenella* on the third day after medication was started. All but three of the treated chicks showed hemorrhage ranging from a trace of blood in the droppings to severe hemorrhage following the *E. tenella* infection but only one of the 12 birds died, whereas all the control chicks showed severe hemorrhage and 8 of the 12 birds died from cecal coccidiosis.

To test further the effect of sodium 4-hydroxy phenylarsonate in a phenolsulphonate-lactose-boric acid preparation, three experiments were conducted on chicks held in brooder houses.

Ninety-eight chicks 9 weeks old were moved to a brooder house and placed in two similar pens. Forty-nine of the chicks received a phenolsulphonate-lactose-boric acid preparation containing sodium 4-hydroxy phenylarsonate at a dosage which gave a concentration of 0.0269% of the arsenic compound in the drinking water. Forty-nine unmedicated chicks served as controls for the experiment. Each of the 98 chicks was infected with approximately 100,000 oöcysts of *E. tenella* on the second day following the beginning of medication. There were no deaths in the treated group while nine of the control chicks or 18.7% died of cecal coccidiosis.

In a similar experiment involving 102 treated and 102 control chicks where sodium 4-hydroxy phenylarsonate was given in a phenolsulphonate-lactose-boric acid preparation at the above concentration, two of the treated chicks (1.9%) and 46 of the control chicks (45.0%) died of cecal coccidiosis.

In a third experiment on a similar preparation where the concentration of the arsenic compound was 0.0475% in the drinking water, there were no deaths from coccidiosis in a group of 101 treated birds while 28 of the 101 control chicks (27.7%) died of cecal coccidiosis. There were few, if any, indications of toxicity in the treated chicks of these experiments.

The introduction of a nitro group into the meta position of the 4-hydroxy phenylarsonic acid nucleus was found to increase the toxicity of the arsonic acid to chickens but also permitted effective use of a lower concentration in the drinking water.

Eight battery-raised chicks 6 weeks old were placed in individual cages for the first experiment on 3-nitro 4-hydroxy phenylarsonic acid. Two of the chicks received this compound at a concentration of 0.0176% in their drinking water; two chicks received this arsonic acid at a concentration of 0.035%; and four unmedicated chicks served as controls. Each chick was infected with approximately 100,000 sporulated oöcysts of *E. tenella* on the third day after medication was started. None of the chicks receiving 3-nitro 4-hydroxy phenylarsonic acid passed any blood following the experimental infection but all four controls showed severe hemorrhage and one of them died of cecal coccidiosis.

In numerous experiments similar to the above, the disodium salt of 3-nitro 4-hydroxy phenylarsonic acid has proven effective in the prevention of clinical symptoms of cecal coccidiosis. Typical results are shown in an experiment where four chicks received disodium 3-nitro 4-hydroxy phenylarsonate at a concentration of 0.0104% in their drinking water. Four chicks receiving no medication served as controls. None of the treated chicks passed any blood in their droppings following an infection with approximately 100,000 sporulated oöcysts of *Eimeria tenella* while all four controls showed severe hemorrhage and three of them died.

Like 4-hydroxy phenylarsonic acid and its sodium salt, the 3-nitro derivatives of the parent compound are compatible with sodium and ammonium phenolsulphonates, lactose and boric acid.

Twenty-four New Hampshire chicks 6 weeks old were placed in individual cages. Sixteen of these chicks received a phenolsulphonate-lactose-boric acid preparation containing 3-nitro 4-hydroxy phenylarsonic acid at a dosage which gave a concentration of 0.0128% of the arsonic acid in the drinking water. Eight control chicks

received no medication. On the third day after medication was started, each chick received approximately 100,000 sporulated oöcysts of *E. tenella*. Only one of the 16 chicks showed hemorrhage following the cecal coccidiosis infection and none died whereas all eight of the controls showed severe hemorrhage and seven died of cecal coccidiosis.

This preparation caused little if any toxicity since the growth of the treated birds was uninterrupted and the preparation was readily consumed. During the 11-day period of the experiment the daily water consumption of the treated and control groups was 101 cc and 88 cc per chick respectively.

An experiment was carried out to determine whether chicks protected by a phenolsulphonate-lactose-boric acid preparation containing 3-nitro 4-hydroxy phenylarsonic acid, while being exposed to large numbers of viable sporulated oöcysts of *E. tenella,* would develop any immunity toward subsequent infection after medication had ceased.

TABLE 1.—*Tabulation of mortality in an efficacy-immunity experiment on chicks receiving 3-nitro 4-hydroxy phenylarsonic acids**

Test group	Description of treatment	Number of chicks	1st infection†		2nd infection‡		Survival at end of the experiment	
			No. infected	No. died	No. infected	No. died	Number	Per cent
I.	Untreated–Uninfected	22	0	0	0	0	22	100.0
II.	Treated–Uninfected	22	0	0	0	0	22	100.0
III.	Untreated–Infected (second infection)	22	0	0	22	15	7	31.8
IV.	Untreated–Infected (twice)	22	22	15	7	0	7	31.8
V.	Treated–Infected (twice)	22	22	0	22	5	17	77.3

* The phenolsulphonate-lactose-boric acid preparation containing 3-nitro 4-hydroxy phenylarsonic acid was given to the chicks of Groups II and V for a period of 10 days at a dosage which gave an arsonic acid concentration of 0.0102% in the drinking water.

† The first infection (approximately 100,000 sporulated oöcysts of *E. tenella* per chick) was given three days after medication was started.

‡ The second infection (approximately 100,000 sporulated oöcysts of *E. tenella* per chick) was given 10 days after the first infection or 3 days after medication was discontinued in Groups II and V.

This experiment was set up according to the 4-pen plan for testing efficacy and toxicity. The experiment as shown in Table 1 was comprised of an untreated-uninfected group (Group I); a treated-uninfected group (Group II); an untreated-infected group (Group IV); and a treated-infected group (Group V). An immunity-control group (Group III) was included for testing the potency of the coccidia culture at the time the untreated-infected and the treated-infected groups were reinfected.

One hundred ten New Hampshire chicks 3.5 weeks old were divided into 5 equal groups using Tippett's table of random sample numbers for assigning the chicks to their respective groups. Groups I, III, and IV of this experiment received no medication and thus served as controls for the experiment while Groups II and V received a phenolsulphonate-lactose-boric acid preparation containing 3-nitro 4-hydroxy phenylarsonic acid for a period of 10 days at a dosage which gave a drinking water concentration of 0.0102% of the arsonic acid.

Three days after medication was started each chick in Groups IV and V received approximately 100,000 sporulated oöcysts of *E. tenella*. None of the treated chicks

(Group V) died as a result of this infection whereas 15 of 22 untreated chicks (Group IV) died of cecal coccidiosis.

On the 10th day after the first infection (3 days after medication was discontinued in Groups II and V) each chick in Groups III and V and the 7 survivors in Group IV received approximately 100,000 sporulated oöcysts of *E. tenella*. Only 5 of the 22 chicks in Group V died whereas 15 of the 22 chicks in the immunity-control group (Group III) died of cecal coccidiosis. None of the 7 chicks in Group IV died as a result of the reinfection. The mortality summary in Table 1 shows that no death occurred in the group of chicks which received doses of *E. tenella* oöcysts similar to that which proved highly fatal to control chicks. At the same time the treated birds developed enough immunity that after they had been taken off medication 77.3% of them survived doses of infective oöcysts which proved highly lethal to susceptible control chicks. The gain in weight made by the treated-uninfected group, during their 21 days on treatment, was 65.5% of the gain made by the untreated-uninfected group.

While testing the safety of 3-nitro 4-hydroxy phenylarsonic acid for young chicks and turkey poults it was found that this compound had remarkable growth-stimulating properties, when given at approximately 25% of the concentration necessary for coccidiosis control. This property of 3-nitro 4-hydroxy phenylarsonic acid is still under investigation and will be more fully reported at some future date.

Certain other aryl arsonic acids have shown some value in the control of cecal coccidiosis but it cannot be accepted as a rule that all aryl arsonic acids are effective. For example, the introduction of an acetyl radical into the amine group of arsanilic acid, while decreasing the toxicity of this compound, greatly reduced its efficacy against *E. tenella* infection.

Like sulfur and many of the sulfa drugs, none of the arsenic compounds tested has shown curative properties against cecal coccidiosis. They are effective only as a preventive treatment. Preventive medication is a recognized practice in human as well as veterinary medicine and it would appear to have a valuable place in the control of avian coccidiosis. Before coccidiosis outbreaks can occur on poultry farms, conditions must be favorable for the sporulation of coccidia oöcysts. Under usual farm conditions a few chicks become infected and gradually spread the disease to other members of the flock. By early recognition of coccidiosis symptoms and prompt application of effective preventive medicine, the writers are convinced that losses from cecal coccidiosis will be greatly reduced.

The writers wish to acknowledge their indebtedness to Messrs. J. P. Henry, A. W. Walde, J. W. Cavett and R. C. Cooper for the preparation and analysis of many of the compounds used.

SUMMARY

1. The two aryl arsonic acids, 4-hydroxy phenylarsonic acid and 3-nitro 4-hydroxy phenylarsonic acid and their sodium salts when given as a preventive in the drinking water of chickens were effective in reducing hemorrhage and mortality from the coccidium *Eimeria tenella*.

2. 4-hydroxy phenylarsonic acid was found to have some effects against the intestinal species of coccidia *Eimeria acervulina*. Non-toxic doses were not effective.

3. While 3-nitro 4-hydroxy phenylarsonic acid prevented mortality from *E. tenella* infection, these birds developed a considerable immunity toward subsequent infection with this species.

PARASITIC INFECTIONS AMONG NATIVES OF THE SAMARAI DISTRICT, PAPUA, NEW GUINEA[1]

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Malariology Unit 66,[2] U. S. Navy

INTRODUCTION

The surveys described herein were primarily intended to ascertain the variety and extent of parasitic infections among natives in order to evaluate the danger of transmission to military personnel within the area.

Seligmann (1901) reported clinical filariasis among natives in British New Guinea. Of possible later surveys in the area, very little literature was available to us. However, the reports of the Allied Malaria Control Conferences were of great assistance. At one of these meetings,[3] Major T. C. Backhouse of the 2/7 Australian General Hospital reported his own surveys subsequent to 1930. He found 19.4 per cent of indentured natives (usually young adult males) from the Mandated Territory to be infected with *Wuchereria bancrofti*. The infection rate for 174 natives from northeastern New Guinea (Morobe to Aitape) was 25.2 per cent. A similar number of natives in New Britain had an infection rate of 7.5 per cent.

Backhouse observed a higher incidence among males than females in the Duke of York Islands. On the island of Makada the rate was 42.6 per cent for males, 26.9 per cent for females. On Maty a similar situation was found: 40.4 per cent for males, 26.2 per cent for females. He (1934) is of the opinion that *Anopheles punctulatus mollucensis*[4] is the vector in this area. He and Dr. G. A. M. Heydon found experimentally that *Aedes aegypti* and *A. scutellaris* were unsuited as vectors in this area. Heydon (1935) proved the culicine, *Aedes* (*Finlaya*) *kochi* to be an excellent experimental vector.

There were no reports of clinical manifestations of filariasis among the 4,000 Europeans of the area included in Backhouse's report, except for very occasional cases of lymphangitis of unknown origin.

The reports on the prevalence of amoebiasis in New Guinea are rather confusing. The medical report (Watch, 1922) for German New Guinea indicates 109 cases there during the year 1909–1910. At another of the Allied Malaria Control Conferences[5] Lt. Col. Bruce Hall recounted the amoebiasis situation in New Guinea. During the Australian Military Occupation, 1914–1920, amoebic dysentery existed but was not common. After 1926, amoebiasis was encountered in the Wau-Bulolo goldfield area. The disease was reported in Finschhafen in 1936–1937. Major Backhouse found a "fair percentage of cyst carriers in natives but saw no actual cases of amoebiasis." A series of 250 fecal specimens from the Mt. Hagen area were found

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[1] The opinions expressed in this article are those of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

[2] The technical assistance of the enlisted personnel of this unit is gratefully acknowledged.

[3] Allied Malaria Control Conference, 43rd Meeting, November 29, 1944, pp. 3–7. [Mimeographed.]

[4] This subspecies may be the same as *A. farauti* of the New Hebrides area.

[5] Allied Malaria Control Conference, 35th Meeting, June 14, 1944, pp. 1–2. [Mimeographed.]

by Dr. Heydon to be negative for *Endamoeba histolytica.* Lt. Col. E. Ford reported that amoebiasis was unknown in the D'Entrecasteaux Group, but a few cases occurred in the adjoining Trobriand Islands.

PROCEDURE

This report is based on two surveys. The first (Series I) was of a small group of adult males comprising an A.N.G.A.U. (Australian New Guinea Administrative Unit) labor camp. This survey was primarily a "pilot run" to ascertain microfilarial periodicity. The second survey (Series II) was made of an entire native village: adults and children.

All results are based on a single examination of a single specimen from each native. Containers for fecal samples were distributed at the time the blood smears were taken and were collected the following day.

For the blood parasites, the thick film technic using Giemsa stain was employed. The slides were examined under low power and the microfilariae, if present, were counted. After these counts were made, the slides were examined for malaria parasites. Stool examinations consisted of a brine flotation for helminth eggs. Fecal smears in physiological saline were examined for protozoa and, where necessary, verified in iodine or water.

The ages of natives, as used in this report, are all apparent ages. They were determined after consultation with the village chieftain and councilors and are believed to be reasonably accurate.

RESULTS

Malaria.—The incidence of malaria among natives of Series I was surprisingly low. The positive smears totaled only 9.5 per cent, all *Plasmodium vivax.*

In series II the incidence was 50.3 per cent. In this series of 318 natives, all three species of malaria were represented. *Plasmodium vivax* predominated; 32.7 per cent of the smears were positive for *P. vivax,* 15.4 per cent for *P. malariae,* 1.9 per cent for *P. falciparum.* In addition there was one mixed *P. vivax* and *P. malariae* and one mixed *P. vivax* and *P. falciparum* infection. Detailed results are included in Table 1.

Filariasis.—To ascertain the existence of microfilarial periodicity, blood smears were taken on the same 63 natives at noon, 7 PM, and midnight. The incidence and density of circulating microfilariae at these times are shown in Table 2.

It should be noted that all individuals positive at 7 PM were also positive at

TABLE 1.—*Results of blood film examinations. Series II*

Natives examined		Number positive			
Age	Number	*P. vivax*	*P. malariae*	*P. falciparum*	*W. bancrofti*
0–4	3	1*	2*	0	0
5–9	59	23	12	3	6
10–14	42	14	8	0	7
15–19	55	16	8	0	18
20–24	48	14	7	0	18
25–29	45	19	3	2	17
30–34	55	18†	6	1†	25
35–39	9	0	4	0	5
Over 39	2	0	0	1	1
Total	318	105	50	7	97

* A mixed infection, *P. vivax* and *P. malariae.*
† A mixed infection, *P. vivax* and *P. falciparum.*

TABLE 2.—*Circulating microfilariae found at specified times in 13 out of 63 natives examined. Series I*

Native number	Microfilariae per slide at the time indicated		
	Noon	7 PM	Midnight
5786	1	16	23
2661	0	6	5
1953	0	88	80
2414	0	43	103
4296	0	22	21
4300	0	18	24
4329	0	42	59
5663	0	12	19
2021	0	0	9
4672	0	0	8
4698	0	0	3
5540	0	0	1
6231	0	0	6
Per cent positive	1.6	12.7	20.6

midnight. The additional positives detected in the midnight series were all light infections. In individuals that were positive on both of the night smears the average number of microfilariae per slide was 36.3. The individuals that were positive only at midnight averaged only 5.4 larvae per slide. Although an effort was made to reduce variation in the size of the blood films, it is realized that these counts represent only an approximate index of microfilarial density.

In Series II, all of the blood smears were taken between 10 PM and midnight. In this group of 318 individuals there were 30.5 per cent positive for microfilariae identified as *Wuchereria bancrofti*. In this second series, the microfilaria count varied from 1 to 524 per slide, with an average of 36.1. Of the 156 males, 32.0 per cent were positive, as were 29.0 per cent of the 162 females.

The incidence of microfilariae was greatest in the highest age group. Specific data on the number and ages of the individuals examined are included in Table 1. Circulating microfilariae were found in individuals as young as 7 years of age.

Intestinal protozoa.—From a military standpoint, the only intestinal protozoan of importance encountered was *Endamoeba histolytica*. The incidence of this parasite in our surveys was very low. In Series I, out of 53 individuals examined, 3 (5.7 per cent) were positive for *E. histolytica*. Of these, 1 was small race *E. histolytica*. In series II, there were 3 positive (1.0 per cent) for this parasite among the 300 specimens examined. All of these were the large race.

Infection rates of the remaining intestinal protozoa were low. Details are given in Table 3.

Intestinal helminths.—Only two species of intestinal helminths were found. In Series I, of 53 persons examined, 1.9 per cent (1 native) were infected with *Trichocephalus trichiurus* and 74.6 per cent with hookworm. In series II, of 300 natives, the infection rate for *T. trichiurus* was 42.0 per cent and 89.3 per cent for

TABLE 3.—*Incidence of intestinal protozoa among New Guinea natives*

Species	Series I			Series II		
	Number examined	Number positive	Per cent positive	Number examined	Number positive	Per cent positive
Endamoeba histolytica ...	53	3	5.7	300	3	1.0
Endamoeba coli	53	2	3.8	300	3	1.0
Endolimax nana	53	1	1.9	300	7	2.3
Giardia lamblia	53	0	0.0	300	1	0.3
Trichomonas hominis	53	1	1.9	300	6	2.0

hookworms. Conditions were such that adult hookworms were not available for specific identification. No tapeworms were detected either by macroscopic or microscopic examinations. No *Ascaris* or *Strongyloides* were found in either series.

DISCUSSION

Malaria.—The percentage of positive smears in Series I was lower than anticipated. As noted above, this series was conducted on a labor camp where medical care was available. Whenever one of these natives was placed on the sick list for any cause he was routinely given 0.6 gm atabrin daily for the entire time he was confined. For most of these natives this procedure resulted in a course of atabrin nearly every month. This may account for the absence of forms other than *Plasmodium vivax* and the low incidence of the latter.

Series II represents a more natural picture of the malaria rate among the natives of the area. There was no atabrin available for these natives except when they became so seriously ill that they were taken to the regional A.N.G.A.U. hospital. Village natives, however, seldom went to the hospital of their own volition.

There appears to be little correlation among malaria rates of the older age groups. As is generally true, the youngest age group had the highest incidence.

Filariasis.—The results of this survey do not indicate a substantially higher incidence of microfilariae among males than females, such as Backhouse found on the Duke of York Islands.

These data show a progressive increase in incidence with increase in age. The youngest individual in which circulating microfilariae were found had an apparent age of 7 years.

Conversations with Australians who have been in the area many years indicate that there were apparently no confirmed causes of clinical filariasis among the pre-war white population of the region. It has been pointed out by others that in areas where microfilariae exhibit nocturnal periodicity there has been little or no transmission to white troops. In fact, elephantiasis among natives of such areas was not seen by us with the frequency or in the grotesque proportions reported in the non-periodic areas. During this survey, numerous cases of enlarged epitrochlear glands were noted. Inguinal enlargements were frequent but not necessarily of filarial origin.

Anopheles punctulatus punctulatus, a probable vector, was very abundant in the area. *Anopheles punctulatus mollucensis* and *Aedes scutellaris* were also collected. *Aedes kochi,* found by Backhouse to be an excellent experimental vector, was present but not common in the area.

Intestinal parasites.—As this survey was conducted under field conditions it was not possible to use routine haematoxylin staining or concentration methods for the detection of intestinal protozoa. Our results, therefore, may indicate a slightly lower incidence than was actually the case.

The eating habits of these natives formed a rather effective control against parasitic infections from food contamination. The food (usually rice, taro, or sweetpotato, and occasionally some meat) was always cooked. The preparation was usually very simple, either by boiling or roasting. No fertilizer was used on their gardens. The food was eaten from individual containers immediately after being

prepared. There was no left-over food problem, and thus the chance of contamination was reduced. Except in the labor camps, food was prepared and eaten by the individual family groups. The custom of thoroughly cooking all meat accounted for the absence of some tapeworms. A majority of these natives chewed betel nut (areca nut) and its taeniacidal effect may also have been a factor. *Ascaris,* although not found during our survey, has been occasionally encountered by A.N.G.A.U. medical assistants.

Excellent pit latrines were provided in the village as were overwater latrines in the labor camp. The high incidence of hookworms, however, indicated that they were not being used. Hookworm infections might easily occur in troops going barefooted along the banks of streams near native villages, as might be the case when they are permitted to swim in such streams.

The difference in the incidence of *Trichocephalus* in the two series is very marked. The camp in which Series I was conducted is located on a coral ledge adjoining a salt-water bay. The village reported on in Series II is located on upland clay soil several miles from the coast. The high salt content and type of soil in the first situation may account for the slight transmission of this parasite. The complete absence of *Strongyloides* is unexplained.

CONCLUSION

Malaria is the only parasitic disease of which there is a serious danger of transmission from natives to white troops in this immediate area. Filariasis among white populations is very rare in areas such as this, where the microfilariae are nocturnally periodic. The transmission of intestinal protozoa is rare under rigid military sanitation programs. Hookworm and *Trichocephalus* are potential dangers, but educational programs for service personnel and regulation of natives within the camp area are effective controls.

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A NOTE ON THE PROBLEM OF PLAGUE IN DAKAR, SENEGAL, FRENCH WEST AFRICA

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The importance of Dakar, as an endemic focus of plague in West Africa, was again emphasized by the recent epidemic in 1944 which took a toll of several hundred lives. Lewis et al (1944) in their report on the outbreak show that during the period from April through November there were 567 cases with a 91% mortality. Records indicate that in the 31-year period from 1914 through 1944 there have been plague outbreaks at Dakar in 18 of those years with a total of 6,562 cases. This gives an average of 364.6 cases per year for the 18 years in which plague actually occurred and an average of 211.7 cases per year for the 31-year period. It should be noted that these case records are necessarily conservative for it is well known that many fatal plague cases among the Negroes of the Dakar region are never recorded since the bodies are secretly buried by relatives motivated by certain economic and social factors the analysis of which is not pertinent to the present discussion.

It was fortunate that American troops completely escaped the ravages of the 1944 plague epidemic. This was due primarily to the strict "off limits" discipline maintained throughout the height of the outbreak. The experience taught us that we must be prepared to deal with plague in Dakar at any time that American troops or nationals may be quartered there.

The report by Lewis et al (1944) has already discussed most phases of the problem. In the present paper we are chiefly concerned with the problem of the flea vector since that aspect was not investigated.

DATA AND DISCUSSION

The presence of plague suggests that the rat flea, *Xenopsylla cheopis,* or some related species is the vector. Data from the L'Institut Pasteur at Dakar (1941) show that *X. cheopis* is the predominant species from rats:

Species	*Host*	*1933*	*1935*	*1936*	*1939*
X. cheopis	Rat	72.9%	71.0%	80.3%	85.7%
E. gallinacea	"	24.0	19.0	11.6	5.4
C. canis	"	2.0	5.0	2.8	5.4
S. pallidus	"	1.1	5.0	5.3	3.5

Notwithstanding these data, it may be possible that a genus other than *Xenopsylla* also has an important relation to the transmission of plague to man in the Dakar region. In this connection it is noted that Negro grass huts and wooden shacks in the Medina (native quarter of Dakar) and in villages surrounding Dakar have a very large flea population which feeds almost exclusively on humans. This

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TABLE 1.—*Fleas collected in native villages about Dakar*

Date	Village	Place	Species*	% of total identified
6–16–44	Medina (Dakar)	Hut floor	*S. pallidus*	100
"	Malika	" "	"	99
			E. gallinacea	1
10–22–44	d'Yof	" "	*S. pallidus*	100
"	N'Gor	" "	"	"
3–23–45	Boun	" "	"	"
"	Kamba	" "	"	"
"	Yombel	" "	"	"
3–25–45	Sangalkam	" "	*E. gallinacea*	99†
			S. pallidus	1
4–1–45	"	" "	"	100
"	Tiaroye Mer	" "	"	"
"	Keur Daouda Sarr	" "	"	"
"	Rufisque	" "	"	"
"	Ouakam	" "	"	"

* All determinations by Dr. Karl Jordan, British Museum. In addition to the above, 10 fleas taken from a mouse in a native hut were all *Xenopsylla brasiliensis* and 7 specimens taken from another mouse in the vicinity of native huts were all *X. cheopis*.

† The predominance of the chicken flea is explained by the presence of many fowls in huts examined.

fact was noted by personnel of an Army malaria survey unit early in 1944 during their visits to native villages in search for anopheline mosquitoes. These troops were attacked by great numbers of fleas within native grass huts and it was observed that the flea attack was no less severe upon the Negro inhabitants.

On this point Lewis et al say the following: "Surveys of the native homes throughout the Medina indicated an enormous and ubiquitous flea population both

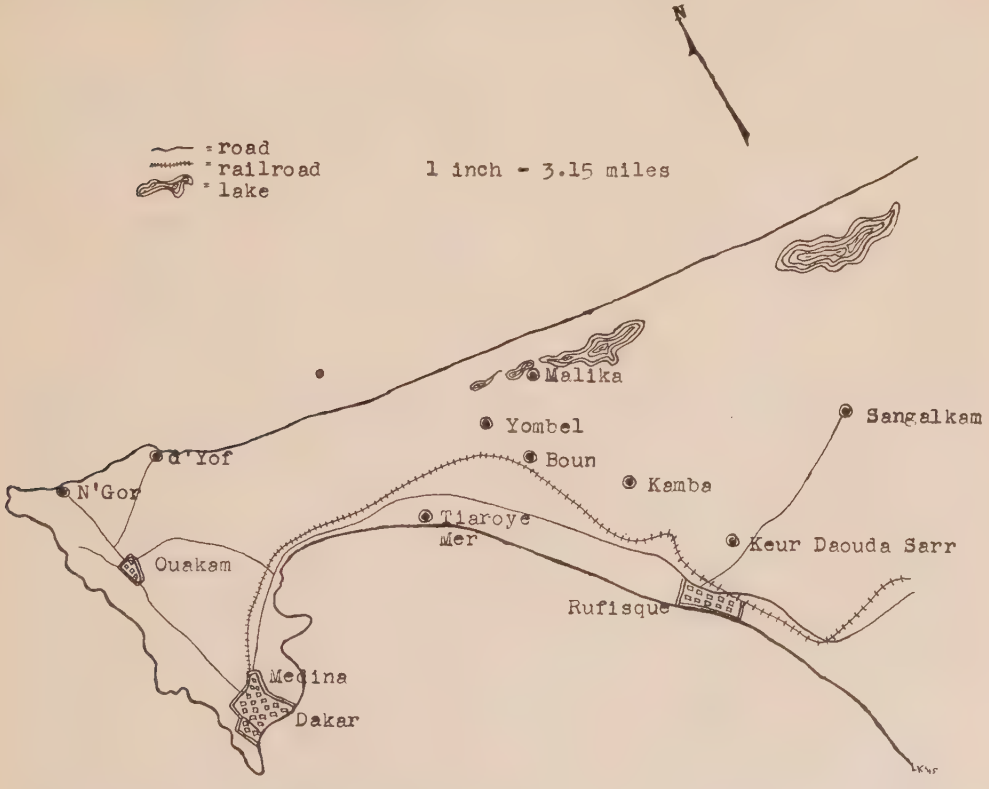

FIG. 1. Native villages affecting the Dakar region in which collections of *Synosternus pallidus* were made.

within the houses and huts—many of which are sand-floored—and on the bodies of the people themselves."

In order to establish an exact identification of the species involved, specimens of fleas from various villages in the Dakar region were sent to Dr. Karl Jordan, British Museum (Natural History), for determination. Dr. Jordan's communications soon made it evident that the predominant "house and body" flea in the Dakar area was *Synosternus pallidus* Taschenberg, 1880. According to Jordan (1945) "*Synosternus* is a genus closely related to *Xenopsylla*. The species *S. pallidus* is known to occur in Northern Africa from Morocco and Northern Nigeria to Egypt and the Sudan and to extend to Palestine, Transcaspia and the Indian Peninsula (Anterior India). Parasitic on rodents, hedgehog (*Erinaceus*) and various Carnivora that prey on these mammals."

Table 1 indicates the prevalence of *S. pallidus* among fleas collected in native grass huts and Fig. 1 shows native villages, affecting the Dakar region, in which collections were made. These data substantially confirm the observations of Ledentu and Peltier (1934) in Dakar. These workers showed that the percentages of the different species of fleas among 7,197 from rodents and 3,181 from earth floors of huts were:

Species	*From rodents*	*From floors*
Xenopsylla cheopis	75.95%	1.10%
Echidnophaga gallinacea	12.85	18.40
Ctenocephalides felis	4.45	0.35
Synosternus pallidus	6.75	80.15

Pasteur Institute data at Dakar (1941) also show a similar distribution of species. During 1939 the percentages of different species of fleas among 13,847 from rats and 8,569 from native huts were:

Species	*From rats*	*From huts*
X. cheopis	85.7%	0.7%
E. gallinacea	5.4	8.4
C. canis	5.4	0.2
S. pallidus	3.5	90.7

In order to gain some idea of the extent of infestation with *S. pallidus* a check on the flea population was made in a native village between 4 May 1944 through 9 July 1944. The check was accomplished by the utilization of sheets of fly paper ("Tanglefoot"), 14 inches long by 8 inches wide. A sheet of fly paper was placed upon the sandy floor of the hut between the operator's feet. The operator then stooped over and waved his hands slowly in all directions but focusing attention just at the center of the sheet (see Fig. 2). This procedure was repeated at three places on the floor—the complete operation lasting 5 minutes—after which the sheet of fly paper with trapped fleas was folded and marked. The fleas from each hut were later counted at the laboratory. Since the same operator checked the fleas throughout the work, probable variations relative to host attractiveness and possible error in procedure were reduced to a minimum.

Table 2 indicates population counts in the village of Malika (see Fig. 1). Population counts could not be made from the center of the plague area since the work was done during the epidemic and control operations had begun. However,

FIG. 2. Method of sampling flea population in native hut.

prior observations on flea infestation together with identification of fleas from various villages suggest that similar results would have been obtained in other villages about Dakar.

Of the 13,059 fleas collected, the males constituted 53.7% and the females were 46.3%. About 40% of this total showed evidence of a recent blood meal, although no attempt was made to determine whether this was human or animal blood. Observations in native huts, which extended over a year, on the feeding habits of these fleas, suggest that they feed almost exclusively on humans. In the Dakar area *Synosternus pallidus* is an extremely domestic species. In this region it seems to be quite as much a specific human ectoparasite as *Pulex irritans* is in other parts of the world. Indeed, it may have usurped this position since no specimens of the human flea were collected in the Dakar region. This may be pertinent to the observation of Bequaert (1930) that while *P. irritans* is common in North Africa, it is not found in the Sahara south of the Atlas Mountains.

These observations naturally raise the question of the probable importance of *S. pallidus* as a vector of plague in the Dakar region. Jordan (1943) has emphasized that "*a priori* every flea is a potential vector." This point is stressed by Lewis

TABLE 2.—*Flea population in the village of Malika*

Date	Collection time per hut	No. huts examined	No. fleas collected	Density of fleas per hut*
5–4–44	5 minutes	5	1,227	245.40
5–6	"	"	1,224	244.80
5–10	"	"	1,232	246.40
5–14	"	"	1,105	221.00
5–18	"	"	1,101	220.20
5–22	"	"	1,066	213.20
5–26	"	"	958	191.60
6–6	"	"	1,278	255.60
6–17	"	"	1,384	276.80
6–23	"	"	1,294	258.80
7–9	"	"	1,190	238.00
5–4–44 to 7–9–44	55 minutes	55	13,059	237.43

* The data cited above would seem to suggest that over 80% of these fleas are undoubtedly *Synosternus pallidus.*

and his co-workers when they say, "It was felt that these house and body fleas were the vectors of the plague within the Medina, as it is questionable that primary pneumonic plague occurred at any time during the outbreak, and as at only one time and at one location were infected rats found in this section—this in spite of the fact that several hundred rats were examined daily from areas surrounding plague cases." Ledentu and Peltier indicate the presence of a rat epizoötic during the 1934 outbreak and say that *S. pallidus* was the dominant species, although they do not definitely suggest it as the vector.

Rat examinations during the period from April through November 1944 show that of 10,500 rats caught, 3,501 were examined in the laboratory and 65, or 1.8% were found to be infected with plague. Since the Pasteur Institute data, cited above, show that *X. cheopis* makes up the largest percentage of the rat-flea population in Dakar, the data on numbers of infected rats during the epidemic would tend to lessen the importance of this species at that particular time.

This is even more strongly emphasized by data on numbers of fleas per rat. In 1939, off 12,259 rats, 13,847 fleas of all species were taken, giving an index of 1.04 fleas per rat. Since *X. cheopis* constituted about 85% of all fleas collected from rats that year, this would give a *cheopis* index of approximately 0.96 fleas per rat. Thus the available numbers of *X. cheopis* are seen to be negligible when compared to numbers of "house and body" fleas (see Tables 1 and 2). It should also be noted that the percentage of *S. pallidus* found on rats is greater than the percentage of *X. cheopis* taken from native huts.

On the basis of circumstantial evidence, we may advance a theory of plague transmission in which *Xenopsylla cheopis* is the primary vector of the plague from diseased rats to man and *Synosternus pallidus* a possible vector which spreads plague from man to man and possibly also from rats to man. Such a combination of circumstances may have obtained in conditions at Dakar during the epidemic of 1944. The absence of primary pneumonic plague, the questionable occurrence of a widespread epizoötic among rats, the negligible incidence of *X. cheopis* in native huts, and the high incidence of *S. pallidus* in native homes all point to the probability that the hypothesis may have some validity. It is clear, at any rate, that more exact observations and experiments on *S. pallidus,* as a potential plague vector in the Dakar region, are indicated.

SUMMARY

The medical importance of Dakar, as an endemic focus of plague, is noted. The 1944 epidemic showed that the United States must be prepared to deal with plague at Dakar at any time that its troops or nationals are quartered there.

Although the rat flea, *Xenopsylla cheopis,* is the most common species on rats, this species is quite negligible in native huts of the villages about Dakar.

The common "house and body" flea in native villages about Dakar is *Synosternus pallidus* Taschenberg, 1880. This is the most domestic species of flea in the Dakar area and feeds almost exclusively on humans. *S. pallidus* is also found on rats in this region and gives a rat-flea index far in excess of the *X. cheopis* index in native homes.

Conditions of the 1944 plague epidemic are cited to indicate that, solely on the basis of circumstantial evidence, it may be probable that *S. pallidus* is concerned in the transmission of plague to humans in the Dakar region.

It is suggested that further study of *S. pallidus,* as a potential vector of plague in the Dakar area, is indicated.

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A NEW ANOPLOCEPHALID CESTODE, *ANDRYA NEOTOMAE*, FROM THE WOOD RAT *NEOTOMA FUSCIPES*

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Thirteen cestodes, which I am describing as *Andrya neotomae* n. sp., were collected on June 23, 1941, from the small intestine of a male *Neotoma fuscipes* caught at the Hastings Natural History Reservation, Monterey Co., California. The worms were fixed in formalin and stained with Ranvier's picro-carmine, Grenacher's borax carmine, or Ehrlich's haematoxylin. The author is indebted to Dr. Harold Kirby for helpful suggestions in the preparation of this paper, and to Dr. Jean M. Linsdale for lending the material from which this study was made.

Andrya neotomae n. sp.

Diagnosis: Scolex diameter average 515 μ, range 355–640 μ; sucker diameter average 221 μ, range 195–249 μ; segments broader than long; genital pores irregularly alternating; 60–74 testes confined to space between ventral excretory ducts, lying dorsal to ovary and on aporal side of proglottid. Cirrus pouch, enclosing seminal vesicle, measuring 320–444 μ in length; vagina expanding into seminal receptacle; ovary and vitelline glands slightly towards poral side; egg diameter 53 μ.

Host: Neotoma fuscipes.

Habitat: Small intestine.

Type locality: Frances Simes Hastings Natural History Reservation, Monterey Co., California.

Type specimens: U. S. National Museum.

Paratypes: Department of Zoology, University of California, Berkeley, California, and Hastings Natural History Reservation, Monterey Co., California.

DESCRIPTION

TABLE 1.—*Measurements of* Andrya neotomae *n. sp.*

Structure	Range	Average	No. of measurements taken
Scolex transverse diameter	355–640 μ	515 μ	13
Suckers transverse diameter	195–249	221	13
Neck length	266–533	353	13
Neck width	162–337	253	13
Mature proglottid length	466–732	586	30
Mature proglottid width	1731–2264	1909	30
Gravid proglottid length	1198–1598	1378	30
Gravid proglottid width	2730–3330	3008	30
Ovary width	284–622	432	28
Cirrus pouch length	320–444	376	30
Cirrus pouch width	89–142	115	30
Testes diameter	35–88	53	40
Ventral excretory ducts width	53–88	68	20

The average length of the specimens in the collection is estimated to be approximately 13 cm. The length of each individual worm could not be established because the fixed material consisted of pieces of various sizes and not of whole individuals. The number of individuals was established by counting the number of scoleces present in the total material. After the worms had been stained and mounted all the parts were measured and the resulting total length divided by the number of scoleces. No measurements of live worms could be taken because the material was already fixed when it reached the author.

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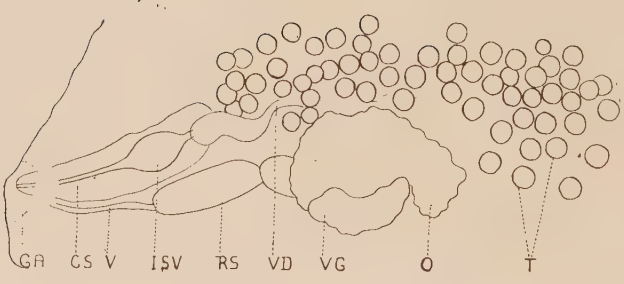
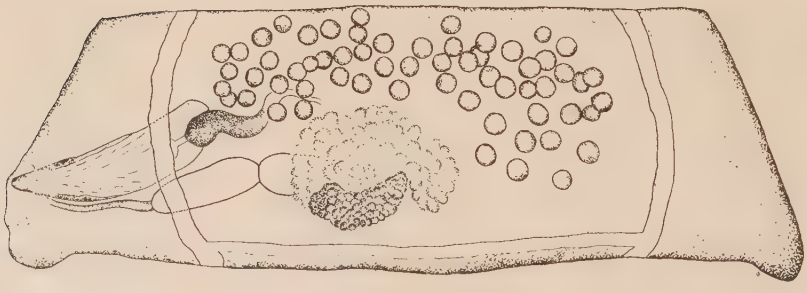
The scolex, bearing four muscular suckers, is followed by a definite neck and then the strobila gradually broadens with the beginning of segmentation. The excretory system consists of a pair of ventral longitudinal ducts with one cross-connection in each proglottid, and of a pair of much narrower dorsal excretory ducts. The number of testes ranges from 60 to 74 in each proglottid (counts made in 30 proglottids). The testes are confined to the space between the ventral excretory ducts and are located anteriorly to the ovary and in the aporal part of the proglottid. The "Anlagen" of the testes appear between the 56th and 60th proglottid, the first proglottid being the first definite segment following the neck region. Clear outlines of the final number of testes appear in the close vicinity of the 80th proglottid. The cirrus pouch is visible in early segments as a darkly staining streak which makes its appearance between the 40th and 45th proglottid. A definite outline of the cirrus pouch is visible in the region of the 80th proglottid. When fully developed the cirrus pouch is seen to contain an unarmed cirrus. The vas deferens is slightly coiled and exhibits an enlarged and darkly staining portion (external seminal vesicle?) before entering the cirrus pouch. Here it enlarges into an internal seminal vesicle. A prostate gland has not been found. The genital pores are situated in the posterior third of the lateral edge of the proglottid. In a series of 48 segments the genital pores were seen to alternate in this fashion: 3,1,1,3,1,2,1,3,1,3,2,1,1,1,3, 1,1,3,2,3,2,3,4,2,—three opening on one side of the strobila, one on the opposite side and so forth. In this series 22 genital pores are situated on one side and 26 on the opposite side of the strobila. In the material studied the largest number of consecutive proglottids having the genital pores situated on the same side, was found to be eight. The vagina opens behind the cirrus pouch into the genital atrium and expands into a voluminous seminal receptacle which enlarges considerably in proglottids between the mature and the gravid stage. The ovary, a much lobed structure, together with the compact and crescent-shaped vitelline gland, is located slightly towards the poral side of the proglottid. The ovary is the first organ to make its appearance in early segments. Its "Anlage" is visible in the very first definite segments following the neck region and it appears as a darkly staining area. A more definite outline of this gland becomes visible in the region of the 60th proglottid and it is fully developed in the region of the 90th proglottid. A clear distinction between ovary and vitelline gland can be made in the region between the 80th and the 90th proglottid. Fully mature proglottids appear after the 90th segment. The uterus becomes visible only in proglottids which are in transition between the mature and the gravid stage. It is at first a diffuse network and then it becomes sacklike and lobed in gravid proglottids. Eggs have a pyriform apparatus and many of them measure 53 μ in diameter.

DISCUSSION

Four species of the genus *Andrya* were recognized by Baer (1927): *A. rhopalocephala, A. cuniculi, A. macrocephala* and *A. primordialis.* They have the following geographical distribution and hosts: *A. rhopalocephala:* Europe; *Lepus timidus. A. cuniculi:* Europe; *Lepus timidus, Oryctolagus cuniculus. A. macrocephala:* North America; *Geomys bursarius. A. primordialis:* North America; *Evotomys gapperi, Sciurus hudsonicus.* As far as morphological features and measurements are concerned *A. cuniculi* resembles the cestode from the wood rat most closely.





EXPLANATION OF FIGURES

All drawings made with the aid of a camera lucida

FIG. 1. Scolex.
FIG. 2. Diagram of genital organs in mature proglottid.
FIG. 3. Mature proglottid.

ABBREVIATIONS

CS: Cirrus sac
GA: Genital atrium
ISV: Internal seminal vesicle
O: Ovary
RS: Receptaculum seminis
T: Testes
V: Vagina
VD: Vas deferens
VG: Vitelline gland

The distribution of the testes appears to be the same and the vagina in both species expands into a large seminal receptacle. The two cestodes differ as follows: in *A. neotomae* the genital pores are situated in the posterior third of the lateral edge of the proglottid whereas they are located in the midline of the lateral edge in *A. cuniculi.* Also the number of testes is different in the two worms. A prostate gland has been described for *A. cuniculi.* Its duct opens into the vas deferens at the point where the latter opens into the cirrus pouch. Although no measurements of this gland are given by Baer, it appears from the drawing that the prostate gland is approximately half the size of the cirrus pouch. No such structure could be found in *A. neotomae. A. rhopalocephala* can be eliminated from our discussion as this species evidently bears little resemblance to *A. neotomae.* The two cestodes show striking differences in size, location of genital pores and number of testes. In *A. macrocephala* the ventral excretory ducts measure 320–430 μ in width, whereas they range from 53 to 88 μ in *A. neotomae.* The testes are confined to the space between the ventral excretory ducts in *A. neotomae,* but extend on the aporal side beyond the excretory ducts in *A. macrocephala. A. primordialis* is set apart from *A. neotomae* by size differences and the number of testes (Baer, 1927). Because of the features discussed the author considers the worm from the wood rat to be a new species.

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CULTURE EXPERIMENTS ON INTESTINAL FLAGELLATES. I. TRICHOMONAD AND OTHER FLAGELLATES OBTAINED FROM MAN AND CERTAIN RODENTS

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I. INTRODUCTION

The culture experiments to be described in these reports were originally undertaken as part of a study of the morphology of trichomonad flagellates. It seemed desirable to test the suggestion made in some accounts in the literature that under cultural conditions the morphology of such flagellates sometimes undergoes alterations which tend to invalidate distinctions among the species. For the species cultivated it can be said that specific characters remained constant during this study.

In culture work on trichomonad flagellates, which apparently do not form true cysts, it is customary to examine cultures and to make transplants at frequent intervals. This method of handling is based upon the widely accepted idea that culture populations decline as a result of the accumulation of noxious waste products as well as to an exhaustion of food supplies. In an effort to save time and labor in the present study, attempts were made to prolong the life of individual cultures by adding nutrients from time to time, and distilled water to replace that lost by evaporation, instead of making so many subcultures. Some of the cultures so treated have remained positive for surprisingly long periods of time.

Another aspect of the work concerns itself with host-parasite relations. Presumably a trichomonad flagellate that lives *in vitro* has advantages in reaching a new host over one that can live only with difficulty outside the host. An intestinal flagellate of a warm-blooded host that survives at room temperature has, theoretically, a better chance of reaching a new host than one that requires temperatures close to that of the host. An intestinal flagellate that can develop in cultures made by adding nutrients to water probably has greater advantages in getting to a new host than one that cannot live in water. These various considerations have determined the nature of the culture work undertaken. In this first report, results of culture experiments on *Trichomonas hominis* and on intestinal flagellates from various rodents, handled up to the end of June, 1945, will be described.

I am indebted to Dr. M. M. Rothman for specimens containing *Trichomonas hominis;* to Mr. LeRoy Saxe for a culture of this species from a dog; to Mr. H. A. Walters for opportunities to examine a Syrian hamster (*Cricetus auratus*) and two pocket gophers (*Thomomys bottae*); to Sharp and Dohme, Glenolden, Pa., and to Dr. H. A. Shelanski, of Smyth Laboratories, Philadelphia, for guinea-pigs; to Dr. Shelanski for the rats from which cultures were made; and to Mr. M. Frumin for a small series of white mice. I am also indebted to the Cerophyl Laboratories, Kansas City, Mo., for a sample of cerophyl, and to the Wilson Laboratories of Chicago for their cooperation in securing a supply of gastric mucin.

Materials and Methods

The cultures were maintained in ordinary test tubes provided with the usual

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cotton plugs. About 10 cc of fluid were placed in each tube. Nutrient materials were primarily gastric mucin (recommended by Ratcliffe, 1934) and Difco Loeffler's dried blood serum, both commonly employed in a concentration of 0.2%. Customarily these were dissolved either in Drbohlav's (1925) modified Ringer's fluid (NaCl, 6 gm; $NaHCO_3$, 0.1 gm; KCl, 0.1 gm; $CaCl_2$, 0.1 gm; distilled H_2O, 1 liter), or in ordinary Ringer's (with 0.8% NaCl) diluted with pond or distilled water. When nutrients were to be added for the maintenance of cultures in single tubes, the Loeffler's serum was usually introduced as the dry powder, or, if fluid was to be restored after reduction by evaporation, it was sometimes dissolved in pond or distilled water. In the earlier part of the study, gastric mucin was commonly dissolved by boiling before it was added. Later, it was introduced in solid form, except when water was to be restored, in which case it was dissolved in distilled water. Commonly the same nutrient was employed throughout the life of any single culture, but in some series, different substances were introduced or alternated with the original in an effort to prolong the survival of the flagellates (Tables 2 and 3). A number of other media were used and will be referred to in the descriptions.

It will be convenient to indicate culture formulae by symbols. R^1 will refer to the customary form of Ringer's solution, while R^2 will indicate the modified form referred to above. L will stand for Loeffler's dried blood serum and M will signify gastric mucin. W will stand for boiled pond or distilled water.

In the tables and descriptions original cultures are indicated by capital letters; subcultures by small letters following a transplant generation number, these two being placed as a subscript under the original culture designation. For example, the second line in Table 1 shows culture $1B_{/3a}$, meaning the first tube (a) of a third transplant generation of the second (B) original culture of strain 1. Total age (column 8, Table 1) is the sum of a) the age of a parent strain at the time a subculture is made, and b) the period of survival of that subculture. In line 2, Table 1, original culture 1B was 61 days old when subculture $1B_{/3a}$ was made from it; survival in this subculture was 69 days, which, added to the 61 days, makes a total age of 130 days.

Since original inoculations were from feces or intestinal contents, the bacteria in the cultures were derived from these original sources. The usual precautions were taken to exclude organisms from the air.

Routinely, a drop of culture from near the surface of the fluid in a tube was examined as well as a drop, with sediment, from the bottom. The flagellate populations were often limited to the bottom layers and were usually denser there, but sometimes more animals were found near the top and occasionally the upper levels were positive when the bottom layers were apparently negative.

II. OBSERVATIONS

Trichomonas hominis

In addition to being able to grow in the incubator, *T. hominis* can also be kept at room temperatures, especially in summer, and can be cultivated in a variety of media. Strains of this species were maintained for a study of their morphology (Wenrich, 1944) and the survival data were obtained from this study. Records are included for 4 strains handled between Aug. 3, 1942, and the end of June, 1943;

and one strain studied in 1945. Four of the strains were from 4 human hosts and one (strain 4) was from a dog. Table 1 presents condensed records of certain of the 166 cultures set up.

As a rule, these flagellates persisted longer at 32–33° C than at room temperatures. The longest survival in a single tube at room temperatures was 69 days (1B/3a) as compared to incubator survivals of 81+ days for strains 1 and 4 and 170+ days for strain 5. This fifth strain did not grow well at room temperature,

TABLE 1

Condensed records of selected cultures and subcultures of 5 strains of *Trichomonas hominis:* 1, 2, 3 and 5 from different human hosts and 4 from a dog. Abbreviations: Cer, cerophyl; cl., clone; E–Me, Difco egg-meat medium; inc., incubator; L, Loeffler's dried blood serum; LiAg, Difco liver infusion agar; M, gastric mucin; R^1, ordinary Ringer's solution; R^2, modified Ringer's (Drbohlav, 1925); r. t., room temperature; W, distilled water.

Cult.	Date of origin	Original medium	Nutrient added	Fluid added	Age at start	Days of survival	Total age	Remarks
1B	Aug. 3, '42	R^2L	..	..	..	21	21	r. t.
1B/3a	Oct. 3, '42	R^2L	2	2	61	69	130	r. t.
1B/4a	Nov. 21, '42	R^2M + LiAg	2	2	110	69	179	inc., 33° C
1B/4b	" 28, '42	R^2L	3	2	117	62	179	inc., 33° C
1B/9b	Mar. 9, '43	R^2L	6	5	218	80	298	inc., 33° C
1B/8c	Apr. 3, '43	R^2L	6	3	233	81	324*	inc., 33° C
1C	Aug. 3, '42	R^2M	3	3	..	61	61	inc., 33° C
1C/5b	Oct. 12, '42	R^2L	1	1	70	33	103	r. t.
1C/6a	" 24, '42	R^2L	1	..	82	42	124	r. t.
1D	Aug. 3, '42	R^2M	..	..	..	21	21	r. t.
1D1/2a	" 29, '42	R^2M	1	..	26	35	61	r. t., cl. 1
1D2/1a	" 12, '42	R^2M	..	..	9	10	19	r. t., cl. 2
1D2/5b	Oct. 24, '42	R^2L	1	1	82	47	129	r. t., cl. 2
2B	Apr. 19, '43	R^2L	..	..	..	4	4	inc., 33° C
2B/2b	" 27, '43	R^2L	6	2	8	31	39	inc., 33° C
3B	May 3, '43	R^2L	..	..	..	8	8	inc., 33° C
3B/3a	" 14, '43	R^2L	4	1	11	36	47	inc., 33° C
4A	Mar. 18, '43	R^2M	..	..	..	10	10	inc., 33° C
4A/1a	" 15, '43	R^2M	2	2	2	29	31	inc., 33° C
4A/2c	Apr. 3, '43	R^2M	7	5	20	81	101*	inc., 33° C
5A	Jan. 2, '45	R^2	10	3	..	49	49	inc., 33° C
5A/1a	" 9, '45	R^2M	40	9	7	170	177†	inc., 33° C
5A/2c	May 1, '45	$1/2R^1$ + Cer	10	3	119	58	177†	inc., 33° C
5A/3a	Feb. 17, '45	R^2M	30	6	46	131	177†	inc., 33° C
5A/5a	Mar. 13, '45	W + E–Me	18	5	70	107	177†	inc., 33° C
5A/4b	" 1, '45	R^2M	2	..	58	10	68	r. t.
5B	Jan. 2, '45	R^2M	..	..	..	1	1	r. t.

* Still positive at end of June, 1943, when cultures were discontinued.
† Still positive at end of June, 1945, when cultures were discontinued.

either in the original culture (5B) or in a transplant (5A/4b), probably because the winter temperatures were too low.

Viability in the different series of transplants from the same strain showed much variation. In original tube 1A in R^2L at 32–33° C, this strain persisted only 5 days and no subcultures were made. Average survival periods (in days) for the other series were: 1B, 45; 1C, 12.7; 1D, 24. In the 1B series a clone group averaged only 12.6 days of survival while the others averaged 56 days. In the 1D series 2 clones had a slightly higher average than the others.

Strains 1, 4 and 5 were definitely more viable than 2 and 3. The average survival time for each in days was: 1, 30.3; 2, 8.7; 3, 15; 4, 32.6 and 5, 94.4. The longest survivals for strains 2 and 3 were 31 (2B/2b) and 36 days (3B/3a), respectively. The true capacity for survival of the cultures set aside in June, 1943, and June, 1945, was, of course, not determined. The rather extensive variations in survival times for the same strain, even when the same medium was used (e.g., 4A/1a compared with 4A/2c) may reflect the absence of any control over the bacterial flora in such cultures.

Trichomonads from Rats and Mice

Elsewhere (1944) I have called attention to the fact that among the trichomonad flagellates of rats there is one which is morphologically and physiologically identical with *T. hominis* of man. Therefore the one from the rat should be as easy to cultivate as the one from man. On Jan. 23, 1945, cultures were inoculated with the caecal contents of 2 rats, freshly killed. The original cultures and all but 2 of the subcultures were kept at room temperatures. Of the 3 originals inoculated from rat 1, two were still positive on June 26, 1945, when they were discontinued, the flagellates having survived for 185 days.

The capacity of these flagellates to live in weaker salt solutions was tested. Original culture 1C contained one-half strength Ringer's with both mucin and Loeffler's. It remained positive as long as the one in modified Ringer's (Rat 1A, Table 2). In a transplant from the one-half strength Ringer's (1/2 RML) culture into one with one-fourth strength Ringer's (Rat 1C/2a), the trichomonads failed to survive. However, at the end of 2 weeks, during which dry mucin was added once, this tube was reinoculated from 1C, and it remained positive until discontinued. During its persistence for 105 days, mucin was added 10 times and distilled water 3 times (Rat 1C/2ar).

For the most part the cultures from rat 2 were handled like those from rat 1 with similar results. The 2 original cultures in R^2ML (Rat 2A, Table 2) and 1/2 R^1ML (Rat 2B) were still positive, June 28, 1945, the flagellates having survived for 187 days.

This strain was similar to the first in adaptation to weaker salt solutions. When culture 2B was 3 weeks old a transplant was made into 1/2 R^1ML but the flagellates lived only 2 days (Rat 2B/1c). Nine days later, however, this same tube was reinoculated from 2B and the trichomonads flourished. They persisted for 125 days, until set aside on June 28, 1945 (Rat 2B/1cr). When culture 2B was 49 days old, a transplant into one-fourth strength Ringer's with mucin was successful, and this remained positive for 107 days, until discontinued (Rat 2B/1d). This strain did not persist in the incubator. Two subcultures, one in R^2ML and the other in 1/2 R^1ML, made when culture 2B was one week old, survived only 3 days at 35–36° C.

A culture in R^2M at room temperature from a third rat containing *T. hominis* did not yield any persisting trichomonads (Rat 3A).

Examination of fresh caecal contents of rats 1 and 2 and of stained slides made from them, showed the presence of *Trichomonas muris* in considerable numbers in both rats. Both also had small numbers of *T. hominis* and a smaller species. Careful study showed the latter to be the one that I had at one time (1924) identified as *T. parva* Alexeieff, 1911 (for illustrations see Wenrich, 1935, fig. 61; and 1942, fig. 176, 9). Later it was found that Alexeieff's *T. parva* was identical with *T. hominis* (see Wenrich, 1944), leaving this small species without a name. I therefore propose for it the new name, *T. wenyoni,* in honor of C. M. Wenyon who provided (1907) the first comprehensive account of the caecal protozoa of mice.

In addition to the three species just named, rat 1 had *Hexamitus pulcher* and *Entamoeba muris.* Rat 2 harbored *T. minuta* and *Chilomastix bettencourti.* *T. muris* from both hosts remained in the cultures in a state of immobility for a week and then disappeared. *Chilomastix* remained active for one day. Only the smaller trichomonads survived beyond the one-week period.

At first it was assumed that *T. hominis* was the only trichomonad persisting in the cultures; but when slight differences in size and behavior among them became noticeable, stained slides were made from the cultures. Then it was discovered that both *T. hominis* and *T. wenyoni* were growing side-by-side in nearly all the cultures, with *T. wenyoni* outnumbering *T. hominis* in some of them.

Cultures from mice yielded less interesting results. Caecal contents from 10 white mice were inoculated into R^2, R^2M, R^2L and 1/2 R^1ML at different times when the mice were examined between Sept. 9, 1944, and Feb. 6, 1945. Although 5 of these mice carried *T. muris,* and 2 had *T. minuta,* only one contained a form of

TABLE 2

Condensed records of selected cultures and subcultures from those inoculated from rats, from a Syrian hamster (Ham.), and from a man after swallowing a culture of the hamster flagellate (Ha/Ho). Abbreviations as for table 1 with the following additions: Bh, Difco beef heart; res. fec., residual fecal mass in glass jar; reinoc., reinoculation; Tryp., tryptone. In column 4, figures in parentheses indicate number of times the substance was added.

Culture	Date of origin	Original medium	Nutrient added	Fluid added	Days of survival	Remarks
Rat 1A	Jan. 23, '45	R^2ML	L(2), M(18)	6	185*	r. t.
" 1A/1a	" 30, '45	R^2ML	L(1), M(15)	4	178*	r. t.
" 1B	" 23, '45	R^2M Bh	Dextrose (1)	..	7	r. t.
" 1C	" 23, '45	1/2 R^1ML	L(4), M(17)	3	185*	r. t.
" 1C/2a	Feb. 27, '45	1/4 R^1M	M(1)	..	...	r. t.
" 1C/2ar	Mar. 13, '45	1/4 R^1M	M(10)	3	105*	r. t. reinoc.
" 2A	Jan. 23, '45	R^2ML	L(3), M(15)	4	187*	r. t.
" 2B	" 23, '45	1/2 R^1ML	L(3), M(13)	4	187*	r. t.
" 2B/1b	" 30, '45	R^2ML	M(1)	..	3	inc., 35–36° C
" 2B/1c	Feb. 14, '45	1/2 R^1ML	M(1)	..	2	r. t.
" 2B/1cr	" 23, '45	1/2 R^1ML	M(11), Cer(1)	2	125*	r. t. reinoc.
" 2B/1d	Mar. 13, '45	1/4 R^1M	L(1), M(7)	3	107*	r. t.
" 3A	Jan. 31, '45	R^2M	.....	..	...	r. t.
Ham. A	Apr. 5, '44	R^2M	M(3)	3	88	inc., 32–33° C
" A/3a	Jun. 15, '44	R^2M	M(14)	10	380*	r. t.
" AR/2b	Apr. 18, '44	R^2M	M(17)	12	438*	r. t.
" AR/4a	May 30, '44	1/2 R^2L	.....	..	33	r. t.
" AR/4b	Sep. 9, '44	1/2 R^2M	M(14)	9	294*	r. t.
" AR/5c	Jun. 3, '44	1/4 R^2L	.....	..	29	r. t.
" AR/5f	Sep. 22, '44	1/4 R^2M	.....	..	...	r. t.
" AR/5g	" 22, '44	1/10 R^2M	.....	..	...	r. t.
" AR/6a	Jun. 5, '44	1/10 R^2M	.....	..	1	r. t.
Ha/Ho1A	" 17, '44	R^2M	M(12)	11	378*	inc. 15 da.
" 1B	" 17, '44	R^2M	M(12), Tryp.(1)	10	378*	r. t.
" 1C	" 17, '44	R^2	M(15)	13	378*	inc. 15 da.
" 1D	" 17, '44	R^2	M(15)	11	378*	r. t.
" 1E	" 17, '44	R^2	.....	..	111	r. t., res. fec.
" 4A	" 21, '44	R^2M	.....	..	11	inc. 11 da.
" 4D	" 21, '44	R^2	L(15)	9	374*	r. t.
" 4E/1a	" 23, '44	R^2L	L(15)	9	372*	r. t.
" 4E/1b	" 23, '44	1/2 R^2L	L(15)	9	372*	r. t.

* Still positive at the end of June, 1945, when cultures were discontinued.

the *T. hominis* type, and none had *T. wenyoni. T. muris* remained alive for a maximum of 6 days, most being in a rounded-up condition. The *T. hominis* type lived for only 6 days in an original culture in modified Ringer's with mucin at room temperature, but in a transplant in the same medium it persisted 24 days.

A Trichomonad from a Syrian Hamster

A half-grown Syrian hamster was found dead in its cage on April 5, 1944. In the caecum were large numbers of trichomonads as well as other caecal protozoa. Smears were fixed and stained and some of the caecal contents was inoculated into R^2M and incubated at 32–33° C. Two days later a small trichomonad appeared in this tube. Stained slides of caecal contents showed great numbers of a large *Trichomonas* similar to *T. muris* and an occasional individual of a very small one

resembling *T. minuta* of rats and mice, and also *Chilomastix* and *Giardia,* but all these died out in the culture. The persisting trichomonad, which was present in very small numbers in the original caecal material, grew abundantly but failed to survive in the original tube during the summer of 1944 (Ham. A, Table 2).

Various transplants were made, both at 32–33° C and at room temperature. Those in the incubator were removed to room temperature on July 2, 1944, but failed to survive through July and August when they were not examined. Several of the transplants at room temperature did survive that summer and continued positive until the end of June, 1945. The 5 surviving cultures remained positive for periods of 189 to 438 days, depending upon the date of origin.

Attempts were made to grow these flagellates in pond water cultures with Loeffler's or mucin but without success. They grew well when R^2 was diluted with an equal amount of boiled pond water (Ham. AR/4b) and also when flagellates from this culture were transplanted to a combination of 1 part R^2 and 3 parts pond water, with Loeffler's as nutrient, but this culture did not remain positive through the summer of 1944 (Ham. AR/5c). They failed to survive in 1/4 R^2 with mucin (Ham. AR/5f) and lived only one day when cultures were made in R^2 one part and pond water 9 parts, with Loeffler's or mucin added (Ham. AR/6a).

To determine whether or not this trichomonad could survive in the human digestive tract, on June 15, 1944, I swallowed a third-generation subculture which had been kept at 32–33° C. The 10 cc of culture were mixed with 90 cc of milk before swallowing. As a control, one cc of the milk-culture mixture was combined with 9 cc of R^2M in a test tube kept at room temperature. In this the flagellates grew and were still persisting, June 30, 1945, having survived for 380 days (Ham. A/3a).

No trichomonads had ever been found in my stools, and during the week preceding the intake of the hamster flagellate, daily examination by direct smears and by culture failed to reveal any trichomonads. Two days after swallowing the culture and milk mixture, the stools became positive by culture for this species and remained so for 4 days, although the flagellates were not observed by direct examination. Since then the stools have been negative for trichomonads.

During the 10 days after the intake of the flagellates, not only were cultures made in tubes at 32–33° C and at room temperature, but in each case the residual fecal mass, which had been collected in a glass jar, was covered with modified Ringer's and left at room temperature as an additional culture. Each day that the tubes were positive the residual fecal culture was also positive, but not afterwards. In 2 of these feces-Ringer's cultures in jars, the trichomonads persisted over the summer and one of them remained positive for 111 days (Ha/Ho1E).

Various subcultures were made from those derived from feces and most survived the summer of 1944. The average survival time of the 20 cultures still positive on June 30, 1945, was 367.5 days; 17 of these were maintained in R^2M, 2 in R^2L and 1 in 1/2 R^2L. It will be understood that nutrients and water were added as needed (see Table 2).

Study of stained slides from the host and from the cultures showed that this small trichomonad was morphologically equivalent to *T. wenyoni* of rats. It is interesting that it grew so readily both at incubator and room temperatures; that it survived nearly 4 months in human feces in Ringer's fluid and more than a year

in each of a series of tube cultures, and that it persisted in the intestine of a human being for 6 days.

A Cecal Flagellate from a Pocket Gopher

On Nov. 25, 1944, a pocket gopher (*Thomomys bottae*), which had died the previous night, was examined and the only protozoan found in its caecum was a species of *Monocercomonoides* Travis, 1932. Four cultures were inoculated: A in R^2, B in R^1, and C in R^2M, all incubated at 35–36° C; and culture D, in R^2M, left at room temperature. The flagellates persisted 9 days in A, 20 in B, and 69 in C (Table 3). Several transplants made at different times from culture C remained positive until set aside, June 26, 1945, the oldest having persisted for 136 days (Goph. C/2a, Table 3). In original culture D at room temperature the flagellates survived for 213 days until discontinued on June 26, 1945. Three transplants into R^2M also remained positive for periods of 209, 194 and 161 days until set aside.

On Dec. 28, 1944, I swallowed 170 cc of milk to which had been added 35 cc of cultures of this flagellate from 4 culture tubes. No flagellates of this type were detected in the stools by direct examination or recovered by culture.

On April 3, 1945, another pocket gopher was examined and again the only caecal protozoan was the species of *Monocercomonoides*. Caecal contents were inoculated into R^2M, and 1/2 R^1M, both at room temperature, but the flagellates did not survive.

Caecal Flagellates from Guinea-pigs

During the winter of 1944–45 attempts were made to cultivate some of the flagellates from the caeca of guinea-pigs. Many experiments were made by a graduate stu-

TABLE 3

Condensed records of cultures and subcultures inoculated from a pocket gopher (Goph.) and from 13 guinea-pigs (G.–p.). Abbreviations as in tables 1 and 2 with the following additions: AmAg, Difco amoeba agar; Ri, rice powder; *Mon., Monocercomonoides hassali* (?); *Ret., Retortamonas caviae; Chilom., Chilomastix intestinalis; T. caviae, Trichomonas caviae,* W, boiled pond water.

Culture	Date of origin	Original medium	Nutrient added	Fluid added	Days of survival	Remarks
Goph. A	Nov. 25, '44	R^2	L(1)	..	9	inc., 35–36° C
" B	" 25, '44	R^1	L(3), Tryp.(1)	..	20	inc., 35–36° C
" C	" 25, '44	R^2M	L(4), M(10)	3	69	inc., 35–36° C
" C/2a	Feb. 12, '45	R^2M	L(1), M(41)	7	136*	inc., 35–36° C
" C/4b	Apr. 14, '45	1/2 R^2M	M(19), Cer(5)	4	75*	inc., 35–36° C
" D	Nov. 25, '44	R^2M	M(18)	4	213*	r. t.
" D/1b	" 29, '44	R^1L	.....	..	...	r. t.
" D/1c	" 29, '44	WL	.....	..	...	r. t.
" D/1d	" 30, '44	1/2 R^2M	.....	..	...	r. t.
" D/1e	Dec. 2, '44	R^2M + AmAg	.....	..	...	r. t.
" D/2a	Jan. 16, '45	R^2M	M(13)	3	161*	r. t.
" D/3a	May 2, '45	1/2 R^1 + Cer.	M(4)	1	55*	r. t.
G.–p. 1A	Nov. 28, '44	R^2M	.....	..	...	r. t.
" 2A	Dec. 4, '44	R^2M	L(2), M(16)	4	204*	*Mon., Ret.*
" 3A	" 8, '44	R^2M	Tryp.(2)	..	14	*Ret.*
" 4A	" 8, '44	R^2M	M(15), Ri(2)	5	200*	*Ret.*
" 5A	" 8, '44	R^2M	L(2), M(5), T(2)	1	98	*Ret.*
" 6A	" 15, '44	R^2	.....	..	4	*Chilom.*
" 7A	" 15, '44	R^2	L(1), M(12)	3	193*	*Ret.*
" 8A	" 15, '44	R^1	L(6), M(7)	2	108	*Ret.*
" 9A	Jan. 15, '45	R^2ML	L(3), M(5), Ri(1)	2	56	*Ret.*
" 9B	" 15, '45	1/2 R^1ML	L(1)	..	2	*T. caviae*
" 10A	" 23, '45	R^2ML	L(2), M(14)	4	154*	*Mon.*
" 10B	" 23, '45	1/2 R^1ML	L(2), M(13)	2	154*	*Mon.*
" 11A	" 31, '45	R^2ML	M(1)	..	5	*Mon.*
" 12A	" 31, '45	R^2M	L(2), M(13)	4	146*	*Mon.*
" 13A	Feb. 6, '45	R^2ML	L(1), M(14)	3	140*	*Mon.*
" 13B	" 6, '45	1/2 R^1ML	L(1), M(14)	3	140*	*Mon.*

* Still positive at the end of June, 1945, when cultures were discontinued.

dent who incubated his cultures at 32–33° C and at 35–36° C. Few of the 42 guinea-pigs examined contained *Trichomonas caviae,* which we wished to cultivate, if possible, by the methods described above. In addition to the cultures in the incubators, I attempted to determine what flagellates would survive at room temperatures in our customary media. Cultures from 13 of these hosts were started, mostly in R^2M or R^2ML (Table 3). The only flagellates that persisted for more than a few days were *Retortamonas* (*Embadomonas*) *caviae* and *Monocercomonoides hassali* (?).

In 6 of the original cultures *Retortamonas* persisted longer than any other flagellate, and for periods of 14 to 200 days. In 3 subcultures this flagellate was the ultimate survivor with positive periods of 118 to 157 days. In 6 more primary cultures, *Monocercomonoides* outlived the other protozoa and persisted for 140 to 154 days; in 3 subcultures for 137 to 157 days. In one original culture (G.-P. 2A, Table 3) the two persisted together for a total of 204 days, until June 26, 1945, when the experiment was discontinued.

There was no significant difference as between the results with stronger (R^2) and weaker ($1/2\ R^1$) salt solutions. For the most part, R^2 was the basic fluid in which survivals were from 0 to 204 days, with an average of 94 days. The average survival time of the 12 cultures that persisted more than 2 weeks was 146 days. One-half strength Ringer's was used 7 times and the average survival time for the 4 "successful" cultures was 149.5 days.

Although the differences are not great, *Monocercomonoides* seems to be somewhat more capable of survival in these cultures than *Retortamonas.* The average longevity of the latter in 12 "successful" cultures, persisting for more than 2 weeks, was 123.3 days; that of 10 "successful" cultures of *Monocercomonoides* was 156.6 days.

From time to time various ingredients other than mucin or serum were added to these cultures, but without appreciable effect. These were: bile salts in 0.1% solution; 0.1% tryptone, cerophyl, and rice powder.

In addition to *Retortamonas* and *Monocercomonoides,* the following protozoa were present in one or more of these 13 guinea-pigs; longest persistence in days in these cultures is given in parentheses: *Balantidium caviae* (11), *Trichomonas caviae* (7), *Chilomastix intestinalis* (21), *Monocercomonas* (*Eutrichomastix*) (?), *Entamoeba caviae* (0).

III. DISCUSSION

Survival of Flagellates in Single Culture Tubes

The literature pertaining to the cultivation of intestinal flagellates is extensive but a full review is not called for in this place. In going over this literature, no description of the method of prolonging the life of cultures by adding nutrients to the contents of a tube, when the population begins to decline, instead of making transplants, has been found, though probably it has previously been employed.

The recent paper by Packchanian (1943) reporting the survival of *Trypanosoma cruzi* and *Herpetomonas musca-domestica* in single culture tubes for periods up to 6 years reveals a surprising degree of viability for these organisms. Franchini (1924) had previously reported persistence of *Herpetomonas pyrrhocoridis* for 6 months to more than 2 years and of *Trypanosoma cruzi* for 6 to 10 months in single tubes. Sullivan (1944) has recorded survival of *T. cruzi* in tubes of citrated guinea-

pig blood for as long as 257 days. These flagellates may form rounded-up and possibly "resting" stages. That different kinds of haemoflagellates have different capacities for survival was shown by Packchanian who indicated that several other species of *Trypanosoma* and 2 species of *Leishmania* persisted only 2 to 4 months in single tubes instead of a matter of years.

From these records in the literature and the culture results described in the present account, it seems obvious that accumulation of waste products is usually a very slow process and that exhaustion of the food supply is a much more important factor in bringing about the decline of culture populations. However, it should be pointed out that the concentration of nutrients in my cultures was relatively low. Initial concentrations of serum and mucin were usually 0.2% and sometimes even less. Higher concentrations would doubtless have produced heavier populations but accumulations of wastes would have been correspondingly greater.

On the other hand, it is possible that some of the substances given off by these animals may be beneficial rather than harmful. This is suggested by the fact that on several occasions reinoculation from the original source into a tube, in which flagellates had failed to survive when first introduced, gave rise to strong and persistent populations. Possibly other changes than those arising from the presence of the animals themselves, were responsible for the more favorable results with these "conditioned" media.

For many of these cultures the ultimate longevity was, of course, undetermined, because they were discontinued at the end of June, 1943, or June, 1945; but one may suppose that inevitably a time would be reached when the accumulation of osmotically active materials, at least, would prove to be intolerable to the flagellates.

Trichomonas hominis

Trichomonic hominis has been cultivated in numerous media by many workers. Ratcliffe (1929) stated that *T. parva* of the rat, which is specifically identical with *T. hominis* (Wenrich, 1944), would grow in every medium that he tried, if there were sufficient bacteria and the pH did not go below 5.0. Some authors have reported relatively long survival in various media. Boyd (1919) recorded persistence of a strain of *T. hominis* from man for 65 days in a fecal suspension in salt solution. Kofoid and Swezy (1923) stated that *Pentatrichomonas ardin-delteili* (= *T. hominis*?) lived for 118 days at room temperature in 10% guinea-pig serum. This is longer than the 81 days of survival recorded above for 2 strains of *T. hominis*, one from man and the other from a dog, but less than the 170 days for strain 5 from man.

T. hominis does not appear to be able to live very long in water supplies or in cultures made by adding nutrients or feces to water. However, certain observations have shown that this species can survive in water for short periods of time; for example; for 24 hours (Hegner, 1928), for 18–35 hours (Simić, 1932), or even up to 3 days for *Pentatrichomonas ardin-delteili* (= *T. hominis*?) (Kofoid and Swezy, 1923). Ratcliffe (1929) reported "development" of *T. parva* (= *T. hominis*?) from rats in tap water or distilled water containing 2% of Loeffler's dried blood serum at 37° C, or in NaCl as low as 0.1% with 0.5% serum. My own experiments with *T. hominis* from man in water cultures gave negative results. However, as reported above, *T. hominis* from a rat lived 107 days in one-fourth

strength Ringer's containing only 0.2% gastric mucin. Survival for 24 hours in drinking water, or for 48 hours in milk (Hegner, 1929), might readily allow for transfers from one host to another, especially within a single household.

Trichomonads from Rats and Other Rodents

As previously noted, I have called attention (1944) to the morphological and physiological evidence indicating that *T. hominis* is able to live in rats. An apparently identical form may also occur in mice but as yet I have found no physiological confirmation of this apparent identity. Very similar trichomonads, with 5 anterior flagella, occur in other rodents. I have observed them in the caecal content of the white-footed mouse (*Peromyscus leucopus*), the field mouse (*Microtus pennsylvanicus*) and the muskrat (*Fiber zibethica*); and Tanabe and Okinami (1940) have reported a similar species of *"Pentatrichomonas"* from a ground squirrel (*Eutamias asiaticus*). It remains for future experiments to determine whether or not these morphologically similar forms from various hosts are also physiologically alike.

Several authors have recorded successful cultivation of *Trichomonas muris* from rats, but the reliability of such reports may justifiably be questioned. In many laboratory-bred rats, one may find heavy populations of *T. muris,* especially if the diet has a high starch content. On the other hand there are 3 smaller species of *Trichomonas* in the rat, including *T. hominis* (*T. parva* Alexeieff), that are usually not numerous and may be overlooked among the much larger numbers of *T. muris.* When caecal material with such mixed populations is placed in culture media, and examined later, trichomonads may persist and multiply. If they are not too closely examined, the impression will be gained that *T. muris* has been cultivated. When these culture flagellates are more carefully studied, however, it may be seen that they are not *T. muris,* which usually persists no longer than a week and then in a rounded-up condition. Ratcliffe (1929) has called attention to this situation and states: "It seems, therefore, that these two species of trichomonads which may live together in the intestines of rats represent two distinct types. The first, *Trichomonas muris,* has not developed in cultures and is quickly affected by changes in the intestinal flora of its host. The second, *Trichomonas parva,* grows readily in cultures and is not influenced to any great extent by changes in the intestinal flora of the rats. Hence it is assumed that *Trichomonas muris* is a much more specialized organism than *Trichomonas parva."* Simić (1933) found that he could cultivate *"T. parva"* (= *T. hominis*) of the rat very readily but that *T. muris* did not grow in cultures.

Of the other two small species of *Trichomonas* in rats, *T. minuta* resembles *T. muris* in general morphology, with minor differences, but is much smaller in size, and I have never been able to cultivate it nor *T. muris.* Morenas (1938) is one of the few observers who have confirmed the existence of the 4 species of *Trichomonas* that I briefly described in 1924. Employing the names that I then used he states that *"T. parva"* was easily cultivated but that he did not succeed in culitvating *T. muris.* One tube containing *"T. parva"* remained positive for 75 days in a gelatin-beef-extract-Ringer's culture covered with vaseline. Since my cultures from rats showed that *T. hominis* and *T. wenyoni* are about equally cultivable, the question arises as to which species Ratcliffe and others have cultivated. It is possible that they were dealing with *T. wenyoni* rather than *T. hominis,* or perhaps, as in my cultures, both were present.

I am inclined to consider the protozoa found in the caecum of the hamster to be identical with those in rats. The great bulk of the population consisted of a *T. muris*-like form. Interspersed were small numbers of flagellates resembling *Giardia muris* (from the small intestine), *Chilomastix bettencourti, Trichomonas minuta* and *T. wenyoni.* This last was so sparse that it would probably not have been discovered had it not appeared in culture. I have found this type in *Peromyscus nuttalli* and the guinea-pig. The ease with which the strains from the rat and the hamster have been cultivated, and the fact that the latter strain could live in the digestive tract of a human being for as long as 6 days, shows a high degree of adaptability. It is possible that there is only one species which is capable of living in a number of kinds of hosts.

Monocercomonoides and Retortamonas

The genus *Monocercomonoides* Travis, 1932, contains species from a wide variety of hosts. They are found in scarab beetle larvae, crane-fly larvae, mole crickets, cockroaches, wood-eating roaches, termites, amphibians and mammals. The last-named group includes such rodents as a white-footed mouse (*Peromyscus nuttalli*), the groundhog (*Marmota monax*), the pocket gopher (*Thomomys bottae*), the guinea-pig and rabbits. However, no reliable reports of its occurrence in man have been made and the one from the gopher failed to infect a human being who swallowed it in culture (p. 46). Species of the genus *Retortamonas* Grassi, 1879 (see Wenrich, 1932) have perhaps an even wider host distribution. They occur in caddis-fly larvae, crane-fly larvae, beetle larvae, mole crickets, cockroaches, termites, amphibians, reptiles and mammals. Among the hosts in the last group are rodents, ungulates, and primates (including man). These widespread host associations suggest a high degree of adaptability making for ease of transfer from host to host. *Retortamonas* forms cysts readily, even in cultures, but I have not identified cysts of *Monocercomonoides.*

It is probably significant that among the caecal protozoa of the guinea-pig, only *Retortamonas caviae* and *Monocercomonoides hassali* (?) responded to the cultural conditions that I provided, including room temperatures. The species of *Monocercomonoides* from the gopher was also readily cultivated at room temperature. I have found that among the various flagellates in Japanese beetle larvae and in some large unidentified tipulid larvae, species of *Monocercomonoides* were the only ones that persisted in culture for any length of time, although *Retortamonas* from the tipulid larvae did survive for a few days. Bishop (1932) and Chen (1934) have reported that *Monocercomonoides orthopterorum* from cockroaches is easily cultivated and I have also found this to be true. *Retortamonas intestinalis* of man develops readily in cultures at room temperature, as pointed out by Bishop (1934) and by Dobell (1935), and I have also recently cultured it at room temperatures. One may assume that the high positive correlation between ease of cultivation and widespread host distribution is a significant one.

IV. SUMMARY

1. Cultures of trichomonad and other intestinal flagellates were maintained in single tubes by adding nutrient materials when populations began to decline, and fluid to replace that lost by evaporation, instead of making so many transplants. Ordinary test tubes with cotton plugs were used. Basic fluid most employed was

modified Ringer's fluid (Drbohlav, 1925) (R^2) or, sometimes, a fractional dilution of ordinary Ringer's (R^1). Nutrients were primarily gastric mucin (M) and Loeffler's dried blood serum (L), usually in a concentration of 0.2%.

2. Five strains of *Trichomonas hominis* were grown: 4 from as many human hosts and one from a dog. Two strains were relatively weak with maximum survivals in single tubes of 31 and 36 days. In one culture of each of 2 of the other strains, *T. hominis* survived 81 days, until set aside in June, 1943. One tube of the fifth strain remained positive 170 days until discontinued at the end of June, 1945.

3. *T. hominis* from the caeca of 2 rats was grown at room temperatures. Original cultures in modified Ringer's containing 0.2% gastric mucin and Loeffler's dried serum (R^2ML) remained positive for 185 and 187 days, until discontinued at the end of June, 1945. Two cultures in 1/2 R^1ML remained positive for the same length of time, until set aside. A transplant from one of these into 1/4 R^1M remained positive for 107 days, until discontinued. *T. muris, T. minuta* and *T. wenyoni* (new name) were also present in these rats. *T. muris* and *T. minuta* did not survive but *T. wenyoni* did, persisting along with *T. hominis* and outnumbering the latter in some cultures.

4. Caecal contents of a Syrian hamster (*Cricetus auratus*) were placed in culture on April 5, 1944. Although forms resembling *T. muris, T. minuta, Chilomastix bettencourti* and *Giardia muris* were also present, the only flagellate that grew in culture was a form like *T. wenyoni* of rats. This flourished at room temperatures as well as or better than at 32–33° C. Some of the early transplants established at room temperature survived 2 months of inattention in the summer of 1944 and persisted until set aside at the end of June, 1945. The oldest, in R^2M, remained positive for 438 days. A culture was mixed with milk and swallowed by the writer in June, 1944. Two days later the stools became positive by culture and remained so for 4 days. Cultures derived from these stools remained positive for 378 days, until discontinued. Several cultures in 1/4 R^2M remained negative but one was positive for 107 days. This species lived only one day in 1/10 R^2M.

5. The caecal contents of a pocket gopher (*Thomomys bottae*) yielded a species of *Monocercomonoides* Travis. In an original culture in R^2M at room temperature it persisted for 213 days, until set aside. Original cultures incubated at 35–36° C did not survive longer than 69 days, but a transplant survived 136 days, until discontinued. This species grew well in 2 out of 3 cultures made in one-half strength Ringer's. It could not be recovered from the feces of a man who swallowed it in culture.

6. Caecal contents of 13 guinea-pigs were cultivated at room temperatures. The only persisting flagellates were *Retortamonas caviae* and *Monocercomonoides hassali* (?). Capacity for survival was approximately equal, both persisting in one tube for 204 days, until discontinued. In 6 primary cultures, *Retortamonas* outlived all others, and in 6 more, *Monocercomonoides* was the surviving form.

7. In all persisting cultures, nutrient materials and water were added as needed. The ultimate longevity of cultures that were discontinued, was, of course, undetermined.

8. I have found trichomonads morphologically indistinguishable from *T. hominis* in a number of rodents besides rats: house mouse (*Mus musculus*), white-footed mouse (*Peromyscus leucopus*), field mouse (*Microtus pennsylvanicus*), and the

muskrat (*Fiber zibethica*). Tanabe and Okinami (1940) reported one from a ground squirrel (*Eutamias asiaticus*). It remains for future experimentation to determine whether or not they are also physiologically similar to *T. hominis* of man and the rat.

9. Tentatively, it is concluded that the caecal flagellates found in a Syrian hamster are identical with those in rats.

10. *Trichomonas muris* and *T. minuta* could not be cultivated. Reports of the successful cultivation of *T. muris* are open to question. *T. hominis* and *T. wenyoni* from rats seemed to be about equally viable in culture, hence doubts arise as to the identity of the *"T. parva"* that various authors have reported as growing in their cultures.

11. Various species of *Retortamonas* and *Monocercomonoides* appear to be easily cultivated. It is suggested that the positive correlation between ease of cultivation and widespread host associations for the species of these two genera may be significant.

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INTESTINAL CANTHARIASIS DUE TO *TENEBRIO MOLITOR*

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Cantharíasis (Osler's "scoleciasis"), or accidental parasitism by coleopteran (beetle) larvae in man, is a rather rare and poorly known entity. The disease is to be distinguished from myiasis and scholeciasis, infection by dipteran (fly) and lepidopteran (moth) larvae, respectively. The rôle played by several species of beetle larvae as intermediate hosts for the tapeworm *Hymenolepis diminuta* has aroused more interest than the primary larval infection itself. That cantharíasis can be a disturbing and persistent illness is illustrated by the case herein reported.

Beetle larvae have been recovered from several parts of the human body. Hinman and Faust (1932), who have summarized the early literature on the subject, report a case of infection of the tonsils by *Tenebrio molitor* larvae, discovered at tonsillectomy. Three centuries ago Tulpius (1652) told of the recovery of beetle larvae from a patient's nose and from another patient's urinary bladder. Bateman (1811) added a case of infection of the umbilicus.

The gastrointestinal tract, however, is the usual habitat of parasitizing beetle larvae (Brumpt, 1927; Hinman and Faust, 1932). Senior-White (1920) reports that in India intestinal infection with larvae of *Onthophagus bifasciatus* (SCARABEIDAE) is common enough to have a vernacular name. Nevertheless the phenomenon of cantharíasis is accidental, there being no obligate coleopteran parasite of man. Beetle larvae in the bowel are in a wholly unnatural environment, and, although they can continue to thrive and develop here temporarily, they must eventually be passed out. Since pedogenesis is not known to occur in these beetle larvae, there is probably no multiplication within the bowel.

Although several species are involved, the beetle usually responsible for gastrointestinal cantharíasis is *Tenebrio molitor,* the dark mealworm, which is commonly found in grains, milled cereals, flour, bran, meals, bread, and meat scrap. The life cycle is completed in one year in this medium (Cotton, 1940), and any stage may be accidentally ingested. Although beetle larvae are considerably more resistant to deleterious influences than are larvae of other types of food-infesting insects, it must be assumed that the usual cereal cooking processes regularly kill them. The danger lies in breakfast foods and precooked cereals.

It is uncertain whether gastrointestinal cantharíasis ordinarily follows the ingestion of the insects in the egg or in the larval form. Senior-White (l.c.) makes the statement, "It is probable that whilst in the intestine the larvae are faecal feeders, but how, and at what stage, they get there . . . is unknown." It seems likely that unmasticated larvae may survive, to be passed eventually by rectum. That late-stage larvae can be present as high in the gastrointestinal tract as the stomach without any known method of maintaining their position there while developing from the egg, is well known from vomitus and post-mortem examinations (Pickells, 1824; Riley and Howard, 1889). On the other hand, when infants pass large numbers of larvae, as in the case here presented, the phenomenon is best explained by the previous ingestion of eggs.

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* 1st Lt., M.C., AUS. The case history is that of a patient of Dr. Paul Beaven of Rochester, N. Y., who was kind enough to bring the case to the writer's attention and to allow its publication. Specific identification of the beetle larvae was confirmed by Dr. Wm. H. Anderson of the U. S. Bureau of Entomology and Plant Quarantine, to whom grateful appreciation is extended.

Knowledge of the pathology and clinical picture of intestinal cantharíasis is limited. There is no evidence to suggest that the larvae become attached to the bowel wall or that they damage it in any way. Melena has not been reported. Although minor symptoms referable to any part of the gastrointestinal tract occur, evidence that disease exists is often first discovered when larvae are noticed in the stool.

Eradication of the infection is a difficult matter. Because cantharíasis is rare in this country and because the infection is eventually self-limited, it is difficult to evaluate therapeutic agents. Purges and enemas are prescribed without confidence. Hexylresorcinol is probably the drug of choice, and, because of the notable lack of harmful side effects, the crystoids may always be given a trial, regardless of the patient's age.

CASE REPORT

The patient, an 8-month-old 18-lb. white boy, was born spontaneously at term weighing 4 lb. 14 oz. He was never breast-fed and was started on precooked cereals at 3 weeks. Now at 8 months he is given pureed soups, vegetables, cereals, and fruits. There have been no previous illnesses. The sibling has always been well and has passed no larvae to the mother's knowledge.

The patient was well until the age of 4 months when he began to vomit frequently between feedings. At 5 months the infant passed a "white worm about an inch long" by rectum. At intervals during the next three months the patient passed several more "worms," all of which looked alike to the mother. Six were recovered; each measured 2.4 cm in length and was identified as a larva of *Tenebrio molitor*. All of the larvae were active when passed, and two were alive at the end of two weeks in a dry air-tight jar.

During this period the infant continued to gain weight. However, he took his feedings poorly, vomited frequently, had long crying spells, and seemed to have "colic" to his mother's eyes. There was no diarrhea or gross melena. No larvae were vomited. The infant was given frequent soapsuds enemas and several doses of Castoria without the elimination of any larvae or the improvement of the patient's signs. Larvae were still being passed three months after the appearance of the first.

After the epidemiology of the infection had been explained to the mother, she made a search for beetles in the dry foods in her kitchen. Adult coleopterans were found in a box of precooked cereal. These adults were keyed out to the TENEBRIONIDAE but were lost before specific identification could be made. Subsequently additional beetles have been found in freshly opened boxes of this brand of precooked cereal.

COMMENT

Because of the age of the patient and the long period over which larvae were passed, it is assumed that infection in this case was initiated by the ingestion of the beetle in the egg stage. The recovery of adult tenebrionid beetles from boxes of the infant's precooked cereal clarified the epidemiology. The ineffectiveness of purges and enemas was demonstrated in this case.

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OBSERVATIONS ON THE TROPICAL RAT MITE, *LIPONYSSUS BACOTI,* AS AN ECTO-PARASITE OF LABORATORY ANIMALS AND SUGGESTIONS FOR ITS CONTROL

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The tropical rat mite, *Liponyssus bacoti,* was originally reported (Hirst, 1913) from the Norway rat, *Rattus norvegicus,* in Egypt. Bishopp (1923) and Ewing (1923) independently published records of its occurrence in the United States. Since that time the species has been widely reported, and there are records from the Northern United States (Riley and Johannsen, 1938) as well as from the Southern States where it is usually conceded to be most abundant.

The mite is an avid blood sucker readily attacking man when an opportunity offers. Much annoyance and irritation, arising from bites of this arthropod, have been experienced by occupants of quarters where a rodent extermination program has been carried out. Hungry mites will travel considerable distances and will willingly substitute man for their normal rat host when the latter has been destroyed. Bishopp (1923) describes the reactions suffered by persons who have been bitten, and attention has recently been redirected to this subject by Anderson (1944) who states that "ratmite dermatitis" is frequently overlooked, being diagnosed as scabies or other skin disturbances. Aside from the dermatitis produced by *Liponyssus bacoti,* there is the grave suspicion that it is a disease carrier. Shelmire and Dove (1931) and Dove and Shelmire (1931, 1932) noted that the distribution of the mite correlated well with the occurrence of endemic or murine typhus, and that "rat-mite dermatitis" was coincidental with this disease in Northern and Eastern Texas. They noted further that the feeding habits and biology of these mites could readily be associated with the known course of the disease in rats in such a way that transmission of the typhus from rat to rat could easily take place. Experimental transmission of a laboratory strain of endemic typhus from one laboratory animal to another, through the mites, was also successfully accomplished by these authors. Rats and guinea pigs were employed, and positive evidence of transmission was obtained in eight instances. Furthermore, some evidence was obtained that hereditary transmission through the egg could occur. This evidence should emphasize the need for seriously considering *Liponyssus bacoti* in the rôle of a typhus transmitter, and as an agent for maintaining the disease in the rodent commensals of man. Certainly the detailed study of a typhus outbreak can not entirely exclude this arthropod in favor of the flea species as a responsible factor.

When an unusually heavy infestation of *Liponyssus bacoti* was discovered on rats, mice and hamsters in the animal house of the Eighth Service Command Laboratory, Fort Sam Houston, Texas, in April 1945, an excellent opportunity presented itself for making some population studies and limited experiments on methods of control. Since the infested animals were intended for experimental work relative to study of disease, and since mites were literally crawling everywhere, there was no time for extended research. The practical question of immediate control came first; life history and other observations were secondary.

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The original source of the infestation was not ascertained. Mites were first noticed on hamsters, which apparently are ideal host animals when large populations are to be produced. In one hamster cage the population of mites increased so rapidly, and to such a high numerical level, that the animal was killed. Although several hours had elapsed before the dead hamster was found and placed in alcohol, the mites still remaining on the body totaled 28,000. This apparently represented only a small fraction of the ecto-parasites which swarmed through the nesting material (wood shavings). The feeding mites engorge rapidly and then leave the animal. They seek a dark, dry place for digestion of the meal, shedding of nymphal skin, and for deposition of eggs. Hence, one explanation for the death of the hamster, possibly the one of choice, is that it was occasioned by exsanguination. This is supported by observations on other animals. The continual stream of hungry mites crawling to the animals, and the mass of engorged specimens leaving at any given time was proof that an enormous population was being fed. Three hamsters, other than the one just mentioned, were killed by these mites when placed in an infested cage. The time required varied, apparently associated with the size of the animal and the time needed to build up a mite population.

A full-grown, white rat placed in a cage from which two dead hamsters had been removed immediately began to lose weight and died after twelve days. An adult, white guinea pig, placed in the same cage, became very emaciated in a short time and died at the end of seven days. Both of these animals demonstrated a progressive blanching of eyes, ears and skin, which could be attributed to a loss of blood. Inoculation of brain and spleen material from this guinea pig into another produced no effects which could explain the loss of animals as a result of disease. To test the theory that exsanguination or a toxic substance injected by the mites, rather than a disease, was the cause for death, a white mouse was next placed in the cage. This animal, a full-grown, healthy male, lived only six hours. The experiment was repeated using a more lightly infested cage. In the latter case, the mouse lived fifteen and one-half hours. A count of the mites present on the body of the latter animal at death exceeded 13,900 specimens. The behavior and appearance of the animals observed favored the explanation that loss of blood brought about death, but the possible effect of a toxic substance cannot be ruled out under the conditions which obtained.

In an attempt to check the effectiveness of DDT powder (10% in talc) as a killing agent, a guinea pig which had just been killed by mites was placed in a gallon jar with its teeming ecto-parasites still attached. The jar was then dusted with four grams of 10% DDT dust, and quickly sealed with filter paper. The filter paper, fastened across the mouth of the jar by a ring of paraffin, permitted entry of air but retained the mites. At the end of twenty-eight hours only 76 live mites were recovered from a total of 21,097 found on the animal. This is a kill of 99.63%. It is believed that this reduction can be ascribed to the effects of DDT, since other tests have shown very little, if any, loss of mites through death from starvation or other causes in the first seventy-two to ninety-six hours after removal from the host. Dove and Shelmire (1931) report that eighteen days must elapse before all mites will have perished from starvation, and that larvae, newly hatched, will survive long periods without benefit of even an initial meal.

In another experiment, 80 ml of well-mixed animal bedding from a heavily infested

cage were placed in each of three quart jars. This bedding material consisting of fine wood shavings contained on the average 7.8 mites per ml. One of the jars had previously been sprayed with 1.6 ml of 2½% DDT, xylene emulsion. The spray had been well distributed over the sides and bottom of the jar and allowed to evaporate, leaving a deposit equivalent to 200 mg per square foot. The bedding placed in the second jar was thoroughly dusted with 0.4 grams of 10% DDT in talc; the container received no treatment. The control jar received an aliquot portion of the bedding, but no toxic agent. All jars were closed by placing a sheet of filter paper over the mouth and applying hot paraffin to the point of junction between glass and paper. This procedure insured a tight container, which would retain the mites yet allow adequate passage of air. When seventy-two hours had elapsed the jars were opened and a count was made of the living mites. The control contained 624 living *Liponyssus bacoti,* and this figure was used as a reference in expressing the rate of kill obtained in the jars treated with DDT. There were 68 live mites in the emulsion-sprayed jar, but none in the one which had received dust. This represents a kill of 89.1% for the emulsion and 100% for the dust as applied under the conditions of this experiment.

In handling the mites during these tests, it was noted that DDT seemed to act as a repellent. A generous ring of dimethyl phthalate was, therefore, applied to the mouth of each experimental container in an effort to retain the mites. As a final precaution against the escape of mites, the jars were also placed in a pan containing kerosene. The dimethyl phthalate was entirely successful as a barrier where control jars were concerned, but it was useless where jars had been treated with DDT dust or spray. In the latter instance the exodus of mites following treatment was immediate and dramatic. Within two hours practically every specimen had crawled across the repellent barrier and down the exterior of the jar into the kerosene. Later, another method was developed for retaining the mites, but repellent action of DDT was still so much in evidence that rapid handling of the material was necessary lest some specimens escape.

Further laboratory observations of the mites, in an attempt to devise better ways of controlling them and in an effort to determine their physical appearance at various stages of development, brought forth a number of interesting facts. Fully engorged females will lay from two to eight eggs each, and these may hatch, under conditions which obtain during June in San Antonio, Texas, in twenty-four hours. Egg-laying as observed here was completed in thirty hours. One female produced eight eggs during this period, all of which hatched within the next thirty-five hours.

The six-legged larvae emerging from the eggs shed their skins in a day or two, and assume the eight-legged nymphal stage (protonymph), even though a blood meal is denied them. This is in keeping with observations made on the common fowl mite, *Dermanyssus gallinae,* but does not exclude the possibility that a blood meal may be taken if available. Unlike the adult, the first nymphal stage has two dorsal plates; it also differs in other minor characters. Older nymphs closely resemble the adult, and may be mistaken for them unless carefully examined.

It was decided, when the need for control of the mite infestation in the animal house became acute, that a large scale application of DDT would be attempted. The probable toxicity of the operation to small laboratory animals could not be thoroughly checked in the time available, but on the basis of some earlier trials with DDT

emulsion in xylene the dosage finally selected seemed safe. The tests referred to indicated that 25 mg of DDT applied externally to adult mice in the form of a xylene emulsion would produce typical tremors, but not death unless applications were repeated several times. Since dust seemed to be a safer vehicle for the DDT than oils or emulsions, the standard larvicide powder (10% DDT in talc) was chosen as the insecticide to be used in treating the infested cages and animals.

All infested animal cages containing bedding were emptied and sterilized by directing a jet of live steam over all surfaces. Old bedding was immediately conveyed to the incinerator, while the fresh bedding (wood shavings) used as a replacement was treated with DDT dust at the rate of 2.7 gm per cage. This was equivalent to 2/3 of an ounce per cubic foot of shavings. After the completion of this operation, the animals (mice and hamsters) were returned to the treated cages. Infested rats and guinea pigs were treated by blowing the DDT dust directly on the animals. Although the cages housing the latter were not sterilized, it was believed that the high dosage of dust, twice the amount in smaller cages, would compensate for the omission. As an added measure, the entire animal house was given a thorough cleaning and walls were sprayed to a height of 6 feet with 5% DDT in kerosene. The solution was applied by power equipment as a fairly coarse spray, which wetted the surface completely, but did not produce droplets or a downward flow of liquid on the wall.

An examination of the animals and cages twenty-four hours after dusting indicated that the treatment had been successful insofar as destruction of mites was concerned. Furthermore, no recurrence of the infestation was noted in the ensuing one and one-half months as evidenced by repeated inspections. In its effect upon the animals, however, the treatment was not entirely satisfactory. Although the larger animals, including hamsters, rats and guinea pigs, were not adversely affected, there was a considerable loss of mice. The mouse colony consisted of 88 breeding females, 22 adult males, and 270 young, distributed in 22 cages. When these were examined twenty-four hours later, there were 30 dead mice; 28 adult females and 2 young. It is interesting to note that all the males, although they were distributed one to a cage, and were, therefore, exposed to any possible variation in dosage, survived the DDT treatment. Although all but 2 of the young mice survived the first forty-eight hours, a total of 166 died during the next two weeks. The only ready explanation for this loss, which fits the peculiar conditions involved, is that DDT was responsible for the 2 deaths which occurred during the first forty-eight hours, whereas the subsequent loss of young mice may have been largely due to lack of proper food following the death of nursing females.

For a period of two weeks following the DDT treatment, the young mice were quite normal in appearance, but were apparently unsuited for laboratory purposes. One group of 8, which had been injected with experimental material nine days after this treatment, was quickly prostrated with typical DDT tremors, and, hence, could not be used as intended. No appreciable loss of mice occurred following the second week, however, and at the end of the third week the young animals were again suitable for routine use.

SUMMARY

In April 1945, a heavy, natural infestation of the tropical rat mite, *Liponyssus bacoti,* occurred on hamsters, rats and mice in the animal house of a medical labora-

tory at Fort Sam Houston, Texas. Hamsters were particularly susceptible, and several were killed. Rats, mice and guinea pigs placed in the infested cages after a dead hamster had been removed died in a short time. Death of these animals apparently occurred as a result of exsanguination rather than disease or the toxic effect of the bites.

A trial application of DDT to small amounts of infested animal bedding (wood shavings) resulted in good control. A general treatment of the animal house based on this information, was effective as a control measure for at least one and one-half months. The treatment appeared to have no ill effects on rats and hamsters, but was toxic to mice. During the first twenty-four hours, 30% of the female mice in the breeding colony died. Although only 2 young mice had died by the third day, a large loss took place during the following eleven days. It is believed that this anomalous result may be largely ascribed to the early loss of females, many of which were nursing young at the time of their death. Adult male mice appeared to be more resistant inasmuch as all survived.

Observations made on living mites while making this study showed that engorged females layed from 2 to 8 eggs each within a thirty-hour period. These eggs sometimes hatched in twenty-four hours or less, and the six-legged larvae, although they had received no blood meal, could transform into first stage nymphs within a day or two.

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A COMPARISON OF THE PERCENTAGE DEVELOPMENT OF A MOUSE STRAIN OF *HYMENOLEPIS* IN ALCOHOLIC AND NON-ALCOHOLIC RATS AND MICE*

JOHN E. LARSH, JR.

Studies with mice (Larsh, 1945) show that alcohol in high concentrations has a detrimental effect on natural immunity to *H. nana* var. *fraterna*. In attempts to discover the mechanism involved in this debilitation, results were obtained which suggested an advantage in using rats as well as mice. Before rats could be used, however, it was necessary first to determine to what extent alcoholization influences their natural resistance to a mouse strain of *Hymenolepis*. Although this had been determined for mice it seemed important to include them in these tests for direct comparisons. The results of these comparisons are presented below and are given at this time especially to prepare the way for the future report on the mechanism of alcoholic debilitation.

METHODS

The white mice were of the same strain used in the previous alcohol work mentioned above, and the rats were from an albino colony established in this laboratory years ago. The animals were raised *Hymenolepis*-free in a room separated from infected stock, and those of the same age were used for experiments after matching according to sex and approximate size. Animals selected for alcohol treatment were given daily doses of the drug by mouth in a concentration of 35 per cent. To prevent toxic reactions, it was found best to give approximately one-half the maximum dose at first and to increase it gradually thereafter. The maximum dose was the amount necessary to produce and maintain unconsciousness for one to three hours and stupor for several hours longer. The dose of alcohol required for this varied a great deal depending on age and size of animal, but for mice it was approximately 0.4–0.45 cc and for rats 3–5 cc. The exact amount in each case was determined more by direct observation of results than by other criteria.

The effect on rats of repeated alcohol treatment was somewhat the same as pointed out previously for mice. Failure to gain normal weight, diarrhea, and loss of the shiny gloss of the coat were the most common observations. The alcoholic rats were usually 10–30 grams lighter than the controls at the end of an experiment. However, in one case (experiment five below), the alcoholic rats averaged only 90 gm, compared with 160 gm for the controls. This alcohol group was an exception in showing definite signs of nutritional deficiency, such as bare areas behind the neck, shaggy coat sparsely haired, reddened areas of the skin, and so forth. The remaining experimental details, including the strain of *Hymenolepis* used, have been given or referred to in the previous report mentioned above.

EXPERIMENTAL RESULTS AND DISCUSSION

Rats and mice of similar age were selected and divided into two groups, alcoholic and non-alcoholic. Those of the former group were given the drug daily for a

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period ranging between 17 and 33 days in the various experiments. Twenty-four hours after the last injection all of the animals were given a test dose of *Hymenolepis* eggs, which had been isolated from fresh fecal pellets just before use. In all cases, the index of infection was based on the number of 93-hour cysticercoids present after infection. Because of the large size of the intestine in the rats it required more than twice the time to count the cysticercoids in them as in mice. This made it more desirable to use small numbers of animals for a single experiment, but in so doing repetition of tests was necessary for statistical reasons. A total of seven experiments was completed at intervals involving 50 rats and 61 mice. Aside from supplying sufficient numbers, this procedure permitted the use of animals of different ages, ranging from 1.6 to 3.0 months.

TABLE 1.—*Comparing the percentage development of* H. nana *var.* fraterna *in alcoholic and non-alcoholic rats and mice*

Exp. No.	No. animals	No. alcohol injections	Age at infection (months)	Infecting egg dose per animal	Ninety-three-hour cysticercoids		
					Average number	Range in number	Percentage development
A. Alcoholic rats							
1	4	30	3.0	2000	18.5	14–26	0.9
2	3	33	2.6	1300	17.0	15–19	1.3
3	3	31	2.3	2000	13.3	10–18	0.7
4	3	17	2.2	1500	13.3	7–19	0.9
5	5	23	2.0	2000	31.8	10–48	1.6
6	4	20	1.8	1500	39.0	25–46	2.6
7	3	18	1.6	1200	19.7	16–24	1.6
B. Non-alcoholic rats							
1	4	0	3.0	2000	4.8	2–8	0.2
2	3	0	2.6	1300	2.7	0–5	0.2
3	3	0	2.3	2000	3.7	2–6	0.2
4	3	0	2.2	1500	9.0	7–12	0.6
5	5	0	2.0	2000	5.0	0–8	0.3
6	4	0	1.8	1500	17.2	14–19	1.1
7	3	0	1.6	1200	6.0	3–10	0.5
C. Alcoholic mice							
1	4	30	3.0	2000	83.0	78–89	4.2
2	4	33	2.6	1300	98.5	61–132	7.6
3	3	31	2.3	2000	128.7	118–140	6.4
4	6	17	2.2	1500	70.8	52–90	4.7
5	8	23	2.0	2000	114.1	71–147	5.7
6	4	20	1.8	1500	111.5	72–153	7.4
7	3	18	1.6	1200	41.3	33–52	3.4
D. Non-alcoholic mice							
1	4	0	3.0	2000	29.3	19–39	1.5
2	4	0	2.6	1300	47.0	40–52	3.6
3	3	0	2.3	2000	27.0	18–39	1.4
4	4	0	2.2	1500	26.3	18–32	1.8
5	7	0	2.0	2000	41.0	31–52	2.1
6	4	0	1.8	1500	46.2	18–80	3.1
7	3	0	1.6	1200	9.7	8–12	0.8

The percentage development of cysticercoids in the alcoholic rats ranged from 0.7 to 2.6, compared with 0.2 to 1.1 for the controls (Table 1). This gives an idea of the higher rate in the alcoholic group. However, the experiments differ in age of animals and number of alcohol injections, which make it necessary to consider each separately. Thus considering the experiments in order, the percentages of development for the alcoholic rats were 4.5, 6.5, 3.5, 1.5, 5.3, 2.4, and 3.2 times greater than for the controls. Therefore, in all but the fourth experiment the differences are rather striking. For some reason the rats in this experiment were unusually resistant to the drug and it was not possible to produce distinct unconsciousness without fatal termination. However, this may not be the explanation for their

lack of the same degree of susceptibility to infection as the other alcoholic rats. since another group (sixth experiment) was equally difficult to render unconscious. In fact, all of the rats were much more resistant than mice, which perhaps was due to more rapid oxidation (McNaught and Pierce, 1939). Regardless of this apparent refractiveness to intoxication, the above results indicate that 35 per cent alcohol in most of the young rats as in the mice somehow lowered the natural resistance to *Hymenolepis*.

The small rate of development in the non-alcoholic rats was expected, since this animal is considered an abnormal host for the mouse strain of *Hymenolepis* (Shorb, 1933). However, the percentages (0.2 to 1.1) were much lower than the average of 3.8 per cent reported by Hunninen (1935) for eight black-hooded rats two months of age. It will be noted that this percentage is higher than the average obtained for the non-alcoholic mice in the present study. This may have been the result of his using a different strain of rat and larger egg doses (3500), but more likely was due to the use of a different mouse strain of the parasite. There is some evidence that differences in infectivity exist among such strains (Larsh, 1943).

The alcoholic mice showed percentages of development ranging from 3.4 to 7.6, whereas those for the non-alcoholic mice ranged from 0.8 to 3.6. Considering individual experiments as above, the percentages for the alcoholic mice and controls in experiment 2 compared more closely than in other tests. In this case the percentages for the alcoholic mice (7.6) was 2.1 times greater than for the controls (3.6). The difference reached its peak in experiment three in which the rate for the alcoholic group (6.4) was 4.6 times greater than for controls (1.4). These results are even more consistent than those of a similar experiment in the previous study (Larsh, 1945). This may have been due to the use of younger animals in the present study and more rigid control on the duration of intoxication. In any event, these results show once more that 35 per cent alcohol, given as above, in some way lowered the resistance to initial infection.

SUMMARY

Seven similar experiments were performed in which a comparison was made of the percentage development of *H. nana var. fraterna* in alcoholic and non-alcoholic rats and mice. In all but one experiment, the alcoholic rats showed percentages of cysticercoid development at least 2.4 times greater than those of non-alcoholic controls. At the same time, alcoholic mice had percentages at least 2.1 times greater than controls in every experiment. These results are interpreted to mean that most young rats as well as mice show a reduced natural resistance to *Hymenolepis* following repeated alcoholization.

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FACTORS AFFECTING THE HATCHING OF OVA OF *SCHISTOSOMA JAPONICUM**

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Because the mature ova of *Schistosoma japonicum* do not hatch within the tissues or the feces of the definitive host, we studied the factors which influence this phenomenon. Faust and Meleney (1924) listed tonicity, temperature, chemical constituents and fermentative agents in the medium as the factors influencing the hatching of these ova.

EXPERIMENTAL RESULTS

1. *Effect of aeration on hatching.*—In order to obtain a large yield of miracidia from fecal material, it was necessary to dilute the feces with water until a large quantity of the putrescible material had been eliminated from the eggs. It was further noted that, whereas hatching usually began within an hour and a half after the eggs had been washed at temperatures ranging from 25° to 31° C. and continued for several hours, after that time few miracidia appeared although a number of eggs contained motile larvae. Renewed hatching could be established under such conditions by the simple expedient of siphoning off the supernatant water and replacing it with a fresh supply. When a bicycle pump was used to aerate the supernatant liquid, which was returned to the flask in which the sediment was retained, there was a prompt emergence of miracidia after none had appeared for several hours. A stream of pure oxygen from a tank bubbled through the water containing ova resulted in the appearance of miracidia after their production had ceased and when the fecal suspension had become very foul.

2. *Effect of temperature on hatching.*—In the preliminary experiments it was observed that washed eggs could be kept in the icebox at 18° C. for as long as nine days without deleterious effects and without the larvae emerging. They readily hatched in the laboratory at temperatures ranging from 25° to 31° C. but at higher temperatures hatching was definitely interfered with. The following experiments were performed.

The soft stool of a patient suffering from schistosomiasis was diluted approximately twenty times with raw, filtered indigenous water and poured through a 40-mesh screen. The sediment was collected and washed three times with large quantities of water and an equal quantity of the final sediment was placed in each of two 50-ml. volumetric flasks. These flasks were filled with rain water and one was placed at 29° C. (air temperature) and the other in a water bath at 37° C. The next day at 8 AM miracidia were noted in the neck of the flask which had been kept at 29° C. but none was present in the other flask. In the first flask miracidia continued to appear until 2 PM. The next morning there were no miracidia in either

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flask and the supernatant liquid of both was siphoned off and replaced with fresh rain water. After two hours miracidia appeared again in the flask held at 29° C. At the end of seven hours none had appeared in the other flask. At this time the flask in the 37° C. water bath was removed and its temperature reduced to 29° C. Miracidia appeared in abundance within one hour.

This experiment was repeated with slight modification using the stool specimen of another patient and with similar results.

We frequently observed the presence of both nonactive and active embryos in the eggs within the tissues of the host. In order to see if conditions could be obtained in the laboratory which would cause these embryos to hatch, we conducted the following experiment: The liver of a wild rat was selected in which there were a large number of motile embryos within the eggshells. The tissue was minced and divided equally between two 250-ml. volumetric flasks. Both flasks were filled with filtered, boiled, raw indigenous water. One flask was kept at 28° C. (air temperature) while the other was placed in a water bath at 37° C. Miracidia appeared in the neck of the first flask after one hour and increased in number during the next three hours. In the meantime, no miracidia appeared in the second flask. At this time the contents of the flask held at 37° C. were divided equally into two flasks, one of which was reduced in temperature to 28° C. Within thirty minutes miracidia appeared within this flask. The flask still remaining in the water bath was removed after an hour and within thirty minutes at 28° C., miracidia made their appearance.

This experiment was repeated with eggs obtained from a second rat; the results of the experiment were the same as in the previous one.

Each time that comparable flasks were placed at 37° and 28° or 29° C., it was noted that the ova that had been at the higher temperature produced more miracidia in a shorter period after the temperature had been reduced to 28° or 29° C. than those in the original flask kept at that temperature. This suggests either that development of the embryo to the hatching stage was hastened by the higher temperature or that the sudden releasing of the inhibiting factor of high temperature allowed the same amount of hatching to take place in a few minutes as occurred in the other flask over a longer period.

3. *Effect of salinity on hatching.*—Several preliminary experiments revealed the fact that salt solutions of the general order of physiologic saline solutions would prevent the hatching of ova and hence the following determinative experiments were performed: A part of the original fecal material used in the experiments on the effect of temperature was divided into two equal portions and placed in 50-ml. volumetric flasks. Solution of sodium chloride was added to the flasks, so that the final concentration was 0.7 per cent. One flask was placed at 29° C. (room temperature) while the other was kept in the water bath at 37° C. Both flasks remained overnight and during the next day and night at these temperatures; repeated examination failed to reveal miracidia although examination of the sediment revealed the presence of motile embryos within the eggs. At this time the supernatant liquid was removed and replaced with fresh saline solution. The flasks were then returned to their original temperatures for seven more hours, at the end of which time no miracidia had appeared. The salt solution was then siphoned off and replaced with rain water; both flasks were kept at 29° C. Miracidia appeared in

the neck of each flask within fifteen to thirty minutes and the production of miracidia continued for several hours.

In a second experiment, in which the final sediment of the fecal material from the second patient was used, flasks were again prepared in like manner, one being retained at 29° C. and the other at 37° C. in a water bath. Miracidia did not appear in the neck of either flask during a period of eighteen hours. After this, the salt solution was siphoned off and replaced with rain water and the flasks were held at 29° C. Approximately one hour thereafter miracidia were observed in the necks of both flasks.

4. *Effect of temperature on miracidia.*—In order to test the effect on miracidia of temperatures higher than those encountered in the natural waters of Leyte, we observed the action of miracidia in a test tube, so arranged in a beaker-water bath that they could be observed constantly with a hand lens (× 10) during the elevation of temperature. Heat was so maintained as to cause an increase of approximately

TABLE 1.—*The effect of sodium chloride on the miracidia of Schistosoma japonicum*

Elapsed time, minutes	Water (rain)	0.20 per cent NaCl	0.66 per cent NaCl	1.00 per cent NaCl	1.30 per cent NaCl
2	Normal activity	Normal activity*	Reduced motility	Majority dead; others slowed down	All dead; on bottom
10			Reduced motility	Few moving slowly	
15			some motionless	Sluggish motion; many on bottom	
20			Majority motionless; two with normal motility	Majority motionless; remainder retarded motion	
40			Many rounded up; remainder retarded motion	Few slowly moving	
55			Many dead and floating	Majority dead and floating	
70			All dead but one	All dead	

* The miracidia immediately attacked *Schistosomophora quadrasi* at the end of the experiment.

0.5° C. per minute until 37.5° C. was reached. The bath was held between 37.0° and 38.0° C. after that. At the beginning the temperature of the water was 27.0° C. Motion of the miracidia was accelerated as the temperature rose and was most rapid at 33.0° C. When the temperature reached 37.0° C. after nineteen minutes fewer organisms were seen and their motion became slower. There was a definite tendency for them to remain near the bottom of the tube, a tendency which persisted until the end of the experiment. After thirty-eight minutes very few miracidia were motile and these were definitely more sluggish than previously, although an occasional one showed nearly normal motility. After one hour only a few remained motile and these were very sluggish. At the seventy-five minute period none exhibited normal motility and the few remaining were all within 0.5 cm. of the bottom of the tube. At the end of 120 minutes only one sluggish miracidium remained. Throughout the experiment a similar tube used as a control at 28° C. always contained normally active miracidia, which remained in the top layers of the water.

5. *Effect of salinity on miracidia.*—Newly hatched miracidia from the feces of a person infected with the trematode were used for the experiment. The organisms

possessed the characteristic motility of the species in the control dish throughout the experiment. Syracuse watch glasses were used and the proper proportions of miracidia in rain water were mixed with a 2 per cent solution of sodium chloride. These mixtures ultimately contained 0.20, 0.66, 1.00 and 1.30 per cent of sodium chloride. Table 1 records the observations up to seventy minutes' exposure, when the experiment was terminated. Approximately thirty miracidia were contained in each dish. Observations were made at a magnification of 15 diameters with a binocular dissecting microscope.

It will be observed that all the miracidia were killed within two minutes in the concentration of 1.30 per cent; they promptly rounded up and sank to the bottom. In contrast, miracidia in the 0.20 per cent solution remained normally viable throughout the experiment, at the end of which they attacked *Schistosomophora* (*Oncomelania*) *quadrasi* readily. In the 0.66 per cent concentration there was slowing down of the activity of the organisms almost immediately and after fifteen minutes the majority appeared to be motionless with most of the remaining ones moving very slowly. In less than an hour nearly all were obviously dead, rounded up and floating on the surface, while at the end of seventy minutes all had succumbed except one miracidium whose motion was extremely sluggish. In the 1.00 per cent solution an effect similar to that in the 0.66 per cent solution was noted. However, the effect was more rapid, many being dead at the end of two minutes and very few remaining even sluggishly motile at the end of ten minutes. All were dead at the end of the experiment.

COMMENT

It is evident that one of the most important factors controlling the hatching of eggs of *Schistosoma japonicum* is the presence of sufficient moisture to insure their survival. It is further evident that the presence of sufficient oxygen is also crucial and this is undoubtedly related to the lack of putrefaction. For this reason, in order to hatch large quantities of miracidia in the laboratory, it is necessary to dilute the stool specimen with large quantities of water and to wash the sediment thoroughly. In nature this process is taken care of by the action of the rains which wash the diluted stool into large quantities of water.

The body evidently has two mechanisms for preventing the hatching of miracidia within its tissues and bowel and conversely, it is necessary to overcome these two inhibitory actions in order for the eggs to hatch. Body temperature is sufficiently high to prevent eggs being hatched either in the tissues or in fecal material within the intestinal tract. In order to obtain hatching the eggs must be reduced in temperature and the optimum appears to be about that of tropical fresh waters. Faust and Meleney apparently did not appreciate the fact that although body temperature was optimal for *development* of the embryo, a lower temperature might be optimal for *hatching*. This has been proved to be the case.

Furthermore, the normal salinity of the body also prevents the hatching of ova and until this is reduced to some unknown maximum, hatching will not take place. In fecal material within the bowel, the ova still will not hatch because of the temperature of the body and in passed feces the salinity is sufficiently high to prevent hatching until dilution takes place although other factors may also play a rôle.

It would appear that the temperature of the water plays a rôle in the activity and

survival of miracidia and that temperatures as great as 37° C. are definitely harmful. The miracidia display their greatest activity at temperatures normally encountered in their natural habitat. Their accelerated motility at higher temperatures apparently exhausts them.

On the island of Leyte there are a number of tidal streams in the Leyte Valley which are periodically salted by the sea. In addition to this, during storms and typhoons, some of the Coastal Plain has been periodically flooded with sea water. Rough tests made of the water from some of these estuaries and coastal swamps indicated a concentration of sodium chloride of from 0.03 per cent to 1.0 per cent. Up to the present time we have been unable to find *Schistosomophora quadrasi* in these areas. It now appears that, if the snail were present in waters containing approximately 0.5 per cent sodium chloride, it is unlikely that miracidia, if present in such waters, would be able to survive long enough to produce infestation of the snails. On the other hand, in concentrations somewhat below this amount, there is no reason to suppose that infestation of snails would not take place if the snails were present.

CONCLUSIONS

1. The ova of *Schistosoma japonicum* require relatively clean water in which to hatch or at least water which has a sufficient quantity of oxygen.

2. Ova will not hatch at mammalian body temperatures but readily hatch at temperatures encountered in tropical waters.

3. Ova will not hatch in the presence of concentrations of sodium chloride as small as 0.7 per cent.

4. Ova of *S. japonicum,* even though they mature, do not hatch aberrantly in the body of the host because of the elevated temperature and the presence of sodium chloride in sufficient concentration to prevent hatching.

5. Miracidia are most active between temperatures of 28° and 33° C. and become sluggish and usually die after thirty minutes' exposure to a temperature of 37° C.

6. Miracidia are killed by exposure of two minutes to a concentration of sodium chloride as great as 1.3 per cent. They are adversely affected within a few minutes in concentrations as low as 0.66 per cent, in which they survive for approximately one hour. After exposure of one hour to a concentration of sodium chloride of 0.20 per cent they are apparently still normal and may infect *Schistosomophora quadrasi.*

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A REDESCRIPTION OF *PHYSALOPTERA LIMBATA* LEIDY, 1856 (NEMATODA: PHYSALOPTERINAE)

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Leidy (1851) described under the name *Spiroptera scalopis canadensis* a single male nematode found in the stomach of *Scalops canadensis* [*S. a. aquaticus* (Eastern mole)] presumably collected near Philadelphia, Pennsylvania. Later, Leidy (1856) renamed this parasite *Physaloptera limbata.* So far as the writer is aware this nematode has never been described by subsequent investigators and Leidy's brief description without figures constitutes the only morphological information concerning this species. Apparently the type material has been lost as Walton (1927) failed to mention *P. limbata* in his revision of the Leidy collection. Molin (1860) listed this parasite under *Species Inquirendae* while Ortlepp (1922) grouped *P. limbata* under "forms with undetermined number of uteri."

While examining numerous prairie moles (*Scalops aquaticus machrinus*) near Madison, Wisconsin, many physalopterids were collected from the stomach and small intestine which apparently are identical with Leidy's *P. limbata.* Other moles and shrews which were collected, including *S. a. machrinoides* (Missouri Valley mole) from Missouri, Indiana, Ohio, Kansas and Iowa; *Parascalops breweri* (hairy-tailed mole) from Maryland, Vermont and New Hampshire; and *Blarina b. brevicauda* (short-tailed shrew) from Illinois and Wisconsin also harbored nematodes of the same species. The occurrence of this parasite was reported briefly in a summary of the Physalopterinae by Morgan (1940, 1941).

Physaloptera limbata Leidy, 1856

Synonomy: Spiroptera scalopis canadensis Leidy, 1851.

Description: Body white in color, slender, tapering anteriorly, reaching greatest diameter near posterior portion. Cuticle thick, loosely annulated, with fine transverse striations. Cuticle reflected over lips (pseudolabia) to form prepuce-like collar. Mouth surrounded by two large triangular lips. Each lip bears two teeth; a prominent single outer tooth (Fig. 1) and an internal membranous tripartite tooth. Each lip also carries a large prominent subdorsal papilla, a similar subventral papilla, and lateral amphid. Two cervical papillae (deirids) present, situated laterally a short distance behind the cephalic extremity. The nerve ring is immediately anterior to, and excretory pore immediately posterior to, the cervical papillae. Esophagus subdivided into a short anterior muscular portion and a longer posterior glandular portion.

Male: Body length 16 to 21 mm, maximum diameter 560–600 microns, width of head 120 microns, width of collarette 200 microns, length of esophagus 4.0 mm, head-nerve ring distance 340 microns, head-cervical papillae distance 420 microns, head-excretory pore distance 460 microns. Spicules unequal (Fig. 2). Left spicule average length 748 microns, right spicule average length 435 microns. Bursa well developed, ventral surface ornamented by longitudual ridges and beads. Four pairs of pedunculated caudal papillae equidistant from each other, two pairs pre-cloacal, two pairs post-cloacal. Immediately in front of cloaca three ventral sessile papillae in triangular row, middle one larger and slightly closer to cloacal margin. Bursa region posterior to cloaca studded with five pairs of ventral sessile papillae. First two pairs in transverse line immediately behind cloacal opening. Third, fourth, and fifth pairs equidistant on tail.

Female: Body length 26 to 31 mm, maximum diameter 800 microns, width of head 180 microns, width of collarette 285 microns, length of esophagus 4.0 mm, head-nerve ring distance 400 microns, head-cervical papillae distance 480 microns, head-excretory pore distance 506 microns. Eggs in uteri, oval 40×26 microns, head-vulva distance 5.7 mm. Vagina (Fig. 3)

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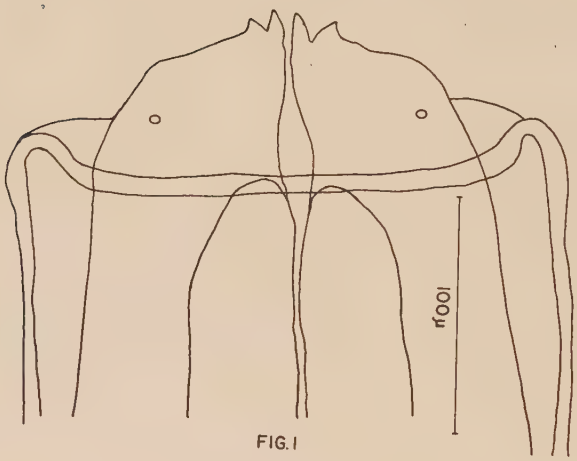

FIG. 1

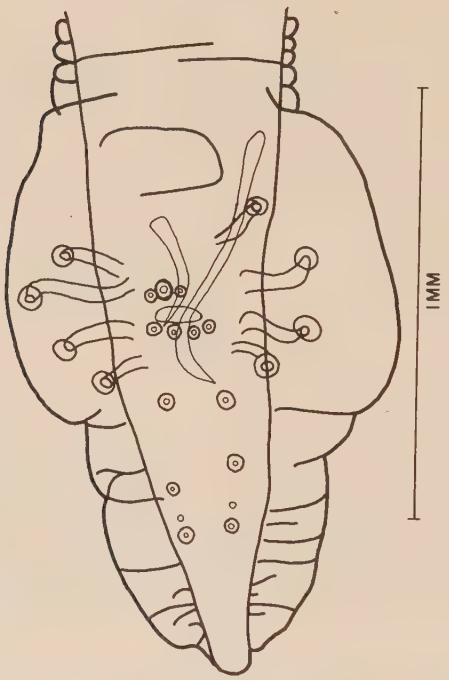

FIG. 2

FIG. 3

EXPLANATION OF PLATE

FIG. 1. Ventral view of anterior extremity.
FIG. 2. Ventral surface of male bursa.
FIG. 3. Portion of female genitalia with vulva, vagina, egg chamber, and common trunk bifurcating to form two uteri.

runs anteriodorsad from the vulva forming a narrow canal 1.4 mm long, 150 microns wide. Distally, vagina widens into egg chamber 480 microns to 1.0 mm long, 240 to 400 microns wide. From the egg chamber there is a common trunk 530 microns to 1.2 mm long which gives rise to two uteri which run in a parallel arrangement to near the posterior end of body and connect with the corresponding oviduct and ovaries.

Hosts: Scalops aquaticus aquaticus (Eastern mole); *S. a. machrinus* (prairie mole); *S. a. machrinoides* (Missouri valley mole); *Parascalops breweri* (hairy-tailed mole); and *Blarina b. brevicauda* (short-tailed shrew).

Habitat: Stomach and small intestine.

Distribution: (U.S.A.) Pennsylvania, Wisconsin, Iowa, Missouri, Illinois, Maryland, Kansas, Vermont, Indiana, New Hampshire, and Ohio.

Specimens: Deposited in the writer's collection and the Helminthological collection, Department of Zoology, University of Wisconsin.

The genus *Physaloptera* contains approximately 42 valid species. The present species belong with a group (*subalata, bedfordi, anomala, muris-brasiliensis, losseni, obtusissima, retusa, papillotruncata, maxillaris* and *limbata*) in which the female possesses the didelphys type uteri with a common trunk and the male four pairs of pedunculated caudal papillae. The species *subalata, alata, bedfordi, anomala* and *muris-brasiliensis* have different numbers and arrangements of sessile papillae than *limbata.* The papillae arrangement is similar in *losseni, obtusissima, retusa, papillotruncata, maxillaris* and *limbata.* However, the reptilian forms, *obtusissima* and *retusa,* may be differentiated from *limbata* by their spear-shaped left spicule, a characteristic not found in the *Physaloptera* from the AMPHIBIA, AVES, or MAMMALIA. The right spicule of *losseni* has a peculiar boomerang shape which distinguishes this species from *limbata. P. papillotruncata* has short equal spicules while those of *limbata* are unequal. *P. limbata* differs from *maxillaris* by having actually and relatively shorter spicules.

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THE EFFECT OF ALCOHOL ON THE DEVELOPMENT OF ACQUIRED IMMUNITY TO *HYMENOLEPIS* IN MICE*

JOHN E. LARSH, JR.

High concentrations of alcohol have been shown recently to have a detrimental effect on the ability of mice to resist an initial infection with *H. nana* var. *fraterna* (Larsh, 1945). The purpose of the present study was to determine the influence of this drug on the development of resistance to a second infection, i.e., its influence on acquired immunity.

Earlier workers have shown that healthy mice are so remarkably resistant to reinfection with this parasite that few, if any, cysticercoids can be demonstrated (Hunninen, 1935; Hearin, 1941). This immunological response is very rapid and appears to be due chiefly to the elaboration of specific antibodies which have been detected both *in vivo* and *in vitro* (Hearin, l.c.; Larsh, 1942, 1943, 1944). Therefore, reinfection of mice following debilitation would reflect an interference with the processes involved in the defense of the body. Because of the importance of learning more concerning this mechanism, it seemed of value to determine whether or not alcoholic debilitation offers such interference. Two experimental approaches were used in this study. In the first the animals were immunized by an initial infection before receiving alcohol, and were reinfected after three weeks of treatment. In other experiments, the mice first were treated daily with alcohol for several weeks and then given an initial infection after which reinfection followed from 24 hours to one week later in the various experiments.

METHODS

Most details of the experimental methods used have been presented previously (Larsh, 1945). It is sufficient to point out that all of the mice used were two months old at the beginning of the experiments, and there were about equal numbers of both sexes. Thirty-five per cent alcohol was given to the experimental mice by mouth in all cases but the dose was not always limited to 0.4 cc as in the previous study. An effort was made to produce and maintain unconsciousness for nearly three hours and stupor from two to four hours longer. While a 0.4-cc dose produced this effect in many cases, it was necessary to give slightly more to some animals. Eggs for infections were isolated from fresh fecal pellets on each day when the period between the first and second infection was one week or longer. Otherwise, the eggs were isolated on the day of the first infection and kept in tap water at room temperature until needed for the second infection.

EXPERIMENTAL RESULTS

The Effect on Acquired Immunity of Alcohol Given Subsequent to Initial Infection

The first experiments were designed to determine whether or not alcohol given several days after initial infection interferes with the immune mechanism. The

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experimental procedure was the same in each of three experiments. Equal numbers of young mice were matched as in the previous study mentioned above and divided into three groups. Those of group A were immunized by an initial infection and five days later given the first of a series of 21 daily alcohol injections. The animals of group B were likewise immunized by an initial infection with a similar number of eggs but alcohol treatment was omitted. This group served, therefore, as a control on the resistance to reinfection of untreated, healthy mice. Group C mice were neither immunized nor alcoholized and served as a control on the viability of the eggs used for reinfection. A similar control was used in each experiment to test the viability of eggs used in the initial infection, but it seemed

TABLE 1.—*Comparing the percentage development of cysticercoids in mice of the same age: (a) immunized by infection and then given a series of daily alcohol injections, (b) immunized but not given alcohol, (c) not immunized and not given alcohol*

Exp. No.	Number of mice	Immunizing egg dose per mouse	Test egg dose per mouse	Ninety-three-hour cysticercoids		
				Average number	Range in number	Percentage development
A. Mice immunized and given 21 daily doses of 35 per cent alcohol						
1	5	840	2000	0	0–0	0.0
2	4	840	1000	0	0–0	0.0
3	6	1300	1000	0	0–0	0.0
B. Mice immunized but not given alcohol						
1	5	840	2000	0	0–0	0.0
2	4	840	1000	0	0–0	0.0
3	6	1300	1000	0	0–0	0.0
C. Mice not immunized and not given alcohol						
1	5	0	2000	48.2	35–58	2.4
2	4	0	1000	20.0	9–29	2.0
3	6	0	1000	18.5	10–28	1.9

unnecessary to give these results, which were typical in all cases. Twenty-four hours after the last alcohol injection was given to mice of group A, all the animals of the three groups were given a similar test infection (Table 1).

A glance at the table shows that not a single cysticercoid was observed in mice of groups A and B, while the number found in those of group C was about that expected from previous studies. It seems clear from these results that with the egg doses used if the body's defenses are uninhibited for five days following initial infection, alcohol given after this time for three weeks has no demonstrable effect on reinfection.

The Effect on Acquired Immunity of Alcohol Given Prior to Initial Infection

The experiments of this section were performed to determine the influence on reinfection of alcohol given daily for several weeks before initial infection. The experimental procedure was identical in all cases. Young mice were divided into two groups, alcoholic and non-alcoholic. Those of the former group were given

the usual daily dose of alcohol for about three weeks before infection. Twenty-four hours after the last dose, these mice and their non-alcoholic controls were divided into various groups for infection. The second infection followed 24 hours, 48 hours, 72 hours, or one week after the first, according to the experiment.

As shown in Tables 2, 3, and 4, the alcoholic mice were divided into two groups, and the non-alcoholic controls into four groups. The alcoholic mice in group one were infected with 200 eggs and at the time of reinfection were given 1600 eggs. Ninety-three hours after the second infection, the animals were sacrificed and a determination made of the number of cysticercoids present from reinfection. Since some of these larval forms may persist in the villi 140 hours or longer after infection (Hunninen, 1935), it was necessary to know whether any remained from the first dose of eggs. This was determined by including a second group of mice. These alcoholic mice in group two were infected with the same number of eggs (200) at the initial infection but were not autopsied until those of group one. Thus, for example, when the second infection followed the first after 24 hours, the cysticercoids in these mice were 117 hours old and not 93 hours as those in group

TABLE 2.—*The percentage development of cysticercoids from a superimposed infection given to alcoholic mice and controls one week after the first infection*

Group	No. mice	No. alcohol injections	Infecting egg dose per mouse		Cysticercoids		
			First infection	Second infection	Average number	Range in number	Percentage development
A. Alcoholic mice							
No. 1	5	20	200	1600	0	0–0	0.0
No. 2	4	20	200	None	0	0–0	0.0
B. Non-alcoholic controls							
No. 1	5	0	200	1600	0	0–0	0.0
No. 2	3	0	200	None	0	0–0	0.0
No. 3	3	0	200	None	8	5–11	4.0
No. 4	3	0	None	1600	16	10–23	1.0

one mice. While such a control seemed unnecessary in experiments where reinfection following initial infection after 72 hours or more, it was used in all experiments for the sake of uniformity.

Groups one and two of the non-alcoholic controls were infected and autopsied at the same time as those of the alcoholic mice. The non-alcoholic mice of group three, given only the initial infection and autopsied 93 hours later, were included as a check on the viability of the eggs used in the first infection. Likewise, the non-alcoholic mice in group four, given only the second infection, served as a control on the viability of the eggs used for reinfection.

1. *The ability of mice to resist reinfection one week after initial infection.*— These results are tabulated in Table 2. No cysticercoids were observed in mice of groups one and two. The eggs for both infections were viable as shown by the presence of 93-hour cysticercoids in mice of groups three and four. The four per cent development of cysticercoids in group three mice is somewhat high for these experimental conditions but was due perhaps to inaccuracy resulting from the use of a very small egg dose. The one per cent development for mice of group four is not unusual. These results show, therefore, that alcohol given daily for three weeks before initial infection with 200 eggs had no demonstrable effect on reinfection with 1600 eggs one week later.

2. *The ability of mice to resist reinfection 72 hours after initial infection.*—This experiment differed from the previous one only in the time interval between infections. The results were almost identical. No cysticercoids were found in mice of groups one and two, and the percentage development for mice of group three was 4.4 and for mice of group four, 0.9. Thus the same conclusion was drawn as in the above experiment, that alcohol given daily for three weeks before initial infection had no demonstrable effect on reinfection 72 hours later.

3. *The ability of mice to resist reinfection 48 hours after initial infection.*—These results differed from those above in that reinfection was demonstrated (Table 3). The percentage development of cysticercoids in group one of the alcoholic mice (0.9) was 30 times greater than that in group one of the controls (0.03), but was not much different from that of group four of the controls (1.4). The counts were not complicated by the presence of cysticercoids from the first infection as none was observed in group two mice. Moreover, these results were not dependent on the lack of viability of eggs used in the first infection as group three mice showed a five per cent development. It seems clear, therefore, that alcohol given daily for

TABLE 3.—*The percentage development of cysticercoids from a superimposed infection given to alcoholic mice and controls 48 hours after the first infection*

Group	No. mice	No. alcohol injections	Infecting egg dose per mouse		Cysticercoids		
			First infection	Second infection	Average number	Range in number	Percentage development
A. Alcoholic mice							
No. 1	5	26	200	1600	14.8	0–46	0.9
No. 2	3	26	200	None	0.0	0–0	0.0
B. Non-alcoholic controls							
No. 1	7	0	200	1600	0.4	0–2	0.03
No. 2	3	0	200	None	0.0	0–0	0.0
No. 3	3	0	200	None	10.0	7–14	5.0
No. 4	3	0	None	1600	23.0	16–28	1.4

26 days before initial infection with 200 eggs interfered with the development of normal resistance to reinfection with 1600 eggs 48 hours later.

It seemed worthwhile to determine the results produced by the same experimental procedure when different combinations of egg doses were used. Ten further experiments were performed, one each week, and a total of 153 mice were used. The number of eggs used for the first and second infections respectively were as follows, with the total number of mice in parenthesis: 700–1050 (16); 500–1250 (16); 550–1650 (24); 150–600 (24); 280–2100 (10); 280–2100 (13); 325–2600 (15); 75–1400 (12); 275–5500 (12); 75–2800 (11). Thus the ratio between the number of eggs used in the first and second infections ranged from 1–1.5 to 1–37.3. When the ratio was less than 1–4 (first three experiments) reinfection 48 hours after initial infection was not demonstrated. At a ratio of 1–4 (fourth experiment) the results were almost identical with those shown above in Table 3. The group one alcoholic mice had an average percentage development of 0.7, compared with 0.03 for their controls. No cysticercoids were observed in group two mice and the number found in those of groups three and four were as expected. The same was true for these three groups in all ten experiments. Ratios of 1–7.5 and 1–8 (experiments five, six, and seven) gave results similar to

those just cited. However, ratios of 1–18.6, 1–20, and 1–37.3 (experiments eight, nine, and ten) produced results which were quite variable. In experiments eight, nine, and ten, the alcoholic mice averaged 0.3, 0.6, and 0.6, respectively, compared with 0.7, 0.1, and 0.4 for the controls. Thus for the combination of egg doses used, ratios less than 1–4 did not result in reinfection, while those between 1–4 and 1–8 gave results similar to those shown in Table 3. The egg dose ratios between 1–18.6 and 1–37.3, on the other hand, produced results that were rather variable and difficult to interpret.

4. *The ability of mice to resist reinfection 24 hours after initial infection.*—Two experiments were performed (Table 4) and the results agree in general with those of the previous experiment. In the first experiment, the alcoholic mice in group one averaged 3.9 per cent development of cysticercoids, compared with 0.8 per

TABLE 4.—*The percentage development of cysticercoids from a superimposed infection given to alcoholic mice and controls 24 hours after the first infection*

Group	Exp. No.	No. mice	No. alcohol injections	Infecting egg dose per mouse		Cysticercoids			
				First infection	Second infection	Total number	Average number	Range in number	Percentage development
A. Alcoholic mice									
No. 1	1	4	25	200	1600	251	62.8	53–71	3.9
	2	5	24	200	1600	232	46.4	29–68	2.9
No. 2	1	3	25	200	None	38	12.7	0–23	6.4
	2	3	24	200	None	0	0.0	0–0	0.0
B. Non-alcoholic controls									
No. 1	1	4	0	200	1600	51	12.8	2–25	0.8
	2	5	0	200	1600	125	25.0	15–38	1.6
No. 2	1	3	0	200	None	0	0.0	0–0	0.0
	2	4	0	200	None	3	0.8	0–2	0.4
No. 3	1	3	0	200	None	18	6.0	5–7	3.0
	2	3	0	200	None	31	10.3	9–12	5.2
No. 4	1	3	0	None	1600	85	28.3	25–32	1.8
	2	3	0	None	1600	204	68.0	59–80	4.3

cent for the non-alcoholic controls in group one. Thus the alcoholic mice had an average percentage development almost five times greater than controls in the same group. However, the three alcoholic mice of group two had an average of 12.7 cysticercoids which remained from the initial infection (117 hours old). It must be assumed, therefore, that each alcoholic mouse in group one likewise harbored approximately the same number. If this assumption is correct, then the total number of cysticercoids shown in the table must not be accredited to reinfection. In other words, 12.7 cysticercoids must be subtracted from the total for each mouse. Thus adjusted, the percentage development for the alcoholic mice (3.1) was still almost four times greater than that for the non-alcoholic controls (0.8). The eggs for both infections were viable as shown by the 3.0 per cent development of cysticercoids in the non-alcoholic mice of group three, and the 1.8 per cent development in mice of group four.

In the second experiment, the alcoholic mice in group one averaged 2.9 per cent development of cysticercoids, while the non-alcoholic controls of the same group averaged 1.6 per cent. These differences were not as great as in the first experiment, but the trend was similar in that the percentages for the alcoholic animals were 1.8 times greater than for the controls. The alcoholic mice of group two in

this experiment showed no cysticercoids, but an average of 0.8 cysticercoid was found in each of the non-alcoholic mice of group two. Adjusting as above, in this case, produced only a trivial change in the percentage (1.5). As above, the eggs for both infections were proved viable by the development of cysticercoids in the non-alcoholic mice of group three (5.2 per cent), and in the mice of group four (4.3 per cent).

The results of these two experiments, although not as striking as those listed in Table 3, tend to show that alcohol given daily for 24–25 days before initial infection with 200 eggs interfered with the development of normal resistance to reinfection with 1600 eggs 24 hours later.

DISCUSSION

The first set of experiments above shows that 35 per cent alcohol given to incapacitate mice for 5–7 hours daily for three weeks after infection does not interfere noticeably with the defenses of the body against *Hymenolepis*. This is in contrast with the influence of a similar treatment on natural resistance (Larsh, 1945). It would appear, therefore, that the defenses are inhibited by alcohol given prior to infection, but, on the other hand, if antigenic stimulation precedes alcoholization such inhibition is not demonstrable. Other studies have demonstrated the inability of alcohol to destroy acquired immunity to certain bacterial and viral organisms (Deléarde, 1897). Although antibodies against *Hymenolepis* are elaborated by mice following infection (*vide supra*), just what part they play in the immunity has not been worked out. Nevertheless, it would be of value to know what effect alcohol has on these protective substances. This, too, is not known in the present case, but has been determined by *in vitro* studies of certain bacterial antibodies (Franzen, 1929). This worker found that an alcoholic concentration up to six per cent actually favored agglutination. If the same obtains *in vivo* for these and other antibodies then it would be impossible to destroy them, since the maximum level of alcohol in the blood compatible with life is usually much less than one per cent (Bogen, 1928; Turner, 1932).

The second set of experiments above shows that alcohol treatment before initial infection has a varying effect on reinfection, dependent upon the time interval between the administration of egg doses. When one week elapsed between the first and second infection none of the alcoholic mice or their controls showed cysticercoids. These results were not surprising because the previous study showed that alcoholic debilitation is temporary and disappears within that time (Larsh, l.c.). The fact that similar results were obtained when the second infection followed the first after 72 hours suggests an even shorter period of debilitation. By giving the second infection 24 or 48 hours after the first, the alcoholic mice showed a much greater reinfection rate than controls. This shows again that untreated, healthy mice respond rapidly to antigenic stimulation, but this was expected in light of knowledge that resistance can be demonstrated in such animals as early as 12 hours after initial infection (Hearin, 1941). Since the alcoholic mice did not respond effectively until sometime between 48 and 72 hours, it seems clear that alcohol in some way influences the rapidity of the immune response. The mechanism involved in this influence is unknown but studies on it are in progress.

A final point of interest is the relation of egg dose size to reinfection. By in-

creasing the ratio between the number of eggs administered at the first infection and the second infection 48 hours later, a point was reached where normal mice allowed the development of unusually large numbers of cysticercoids. This success in increasing the reinfection rate of normal mice may mean that there is a definite limit to the amount of antigen that can be acted upon by antibodies produced in response to the first infection. Whether or not there is such a quantitative relationship between the antigen-antibody combination was not studied further, but others have shown conclusively by *in vitro* methods that the mechanism operates for type III pneumococcus (Heidelberger and Kendall, 1929, 1935a, 1935b).

SUMMARY

Experiments were carried out to determine the influence of 35 per cent alcohol on reinfection of mice with *H. nana* var. *fraterna*. When daily treatment with the drug was not begun until several days after initial infection, it had no demonstrable effect on reinfection several weeks later. Thus the action of the immune mechanism stimulated before alcoholization was not interfered with in degree to be measured by the infection method employed. In other experiments in which the drug treatment was given daily for a few weeks before the first infection the results varied with the time interval before reinfection. When this period was one week or 72 hours, reinfection was not demonstrated. This probably meant that the alcoholic mice had recovered from debilitation. On the other hand, reinfection was demonstrated where only 48 or 24 hours elapsed between infections. In both cases the alcoholic mice showed percentages of cysticercoid development many times greater than those of controls. This indicated, therefore, that alcohol treatment caused a delayed response to antigenic stimulation but the mechanism for this is as yet unknown.

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PLASMODIUM LACERTILIAE N. SP., AND OTHER SAURIAN BLOOD PARASITES FROM THE NEW GUINEA AREA

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Blood smears recently obtained from approximately 60 specimens of *Leiolopisma fuscum* procured from Goodenough Island (in the D'Entrecastaux Island Group) contained the following parasites: *Plasmodium lacertiliae* n. sp., unidentified haemosporidia and haemogregarines, *Haemogregarina, Trypanosoma,* and microfilariae. Several air-dried smears stained by Wright's method were examined from each lizard and most of the lizards were infected with one or more of these types of parasites.

PLASMODIUM

Several low-grade infections of the same malarial parasite were encountered. A comparison of these organisms with descriptions of known species of saurian parasites revealed that they are most nearly like *P. tropiduri* (Aragão and Neiva, 1909) but that they differ from the descriptions and illustrations of this species. In addition we have observed two distinct differences between the parasites of *L. fuscum* and *P. tropiduri* in a direct comparison of smears containing them.[1] The parasites in *L. fuscum* form much larger gametocytes and asexual stages of a more delicate appearance, due to the smaller size of the chromatin masses and the more lightly staining cytoplasm, than corresponding stages of *P. tropiduri.* The differences between these parasites, which are summarized in table 1, seem sufficient to warrant a description of the malarial parasites in *L. fuscum* as a distinct species, and it is proposed that they be referred to as *P. lacertiliae* n. sp. We recognize the disadvantages of describing a new species of parasite simply from the study of stained blood smears from natural infections but the difficulty of securing living parasites from this remote area for study in the laboratory seems to justify the tentative identification of these organisms on this basis.

TABLE 1.—*Comparison of characters of* P. tropiduri *and* P. lacertiliae *n. sp.*

	P. tropiduri	*P. lacertiliae* n. sp.
Position in host cell	Polar	Varies, usually polar
Effects upon host cell	Slight hypertrophy. Nucleus displaced	Slight hypertrophy. Nucleus displaced
Shape and size of gametocytes	Round or indented by erythrocyte nucleus. Macrogametocytes 6–8 μ. Microgametocytes max. 9 μ	Irregularly oval, indented by erythrocyte nucleus. Macrogametocytes 6.9×12.0 μ. Microgametocytes 5.8×9.2 μ
Pigment granules	Fine, round	Fine, round
Shape of schizonts	Round or oval	Round or elongate oval
Number of merozoites	Maximum 12	6–15, average 10
Type host	*Tropidurus torquatus*	*Leiolopisma fuscum*
Type locality	Minas Geraes, Brazil	Goodenough Island (New Guinea Area)

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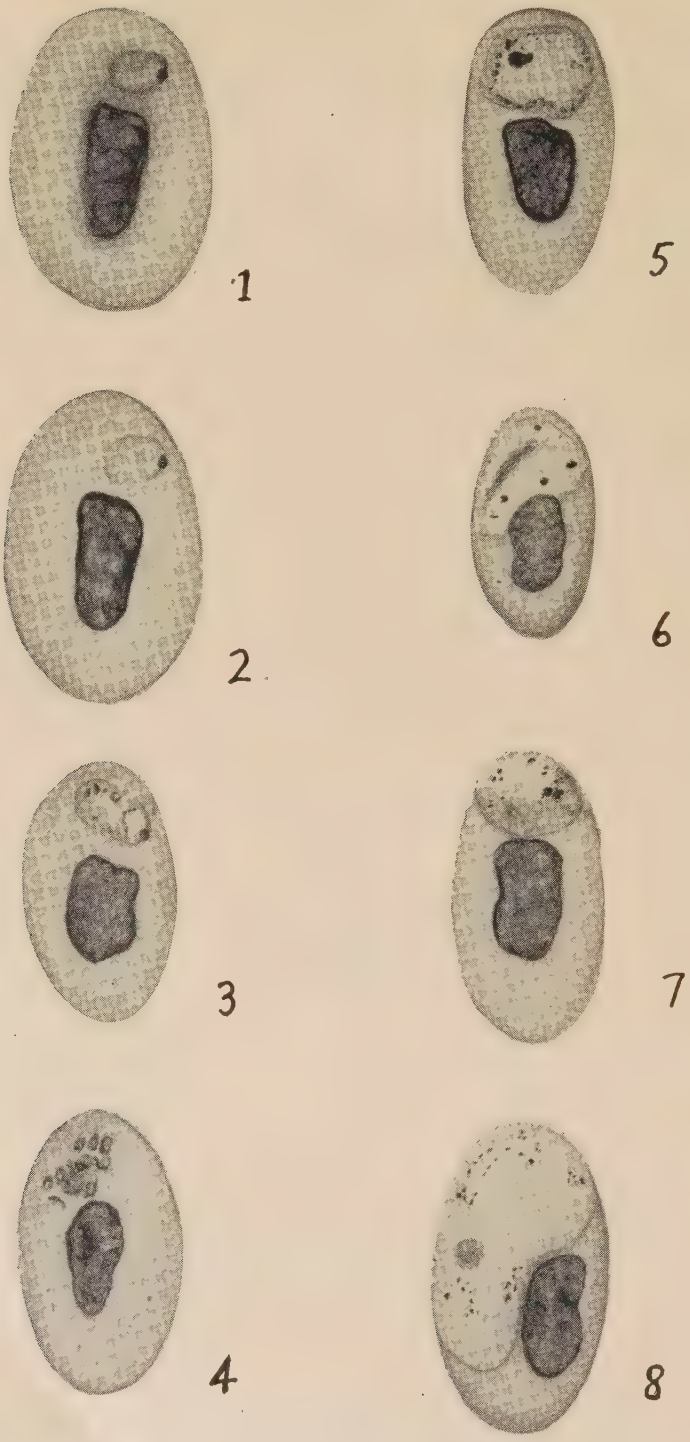

EXPLANATION OF PLATE

Plasmodium lacertiliae n. sp. All figures drawn with the aid of a camera lucida. Magnification × 2000.

FIGS. 1–2. Trophozoites. FIG. 3. Immature schizont. FIG. 4. Mature schizont. FIG. 5. Immature microgametocyte. FIG. 6. Mature microgametocyte. FIG. 7. Immature macrogametocyte. FIG. 8. Mature macrogametocyte.

Plasmodium lacertiliae n. sp.
(Figs. 1–8)

The parasites occupy various positions in the cytoplasm of erythrocytes. Asexual stages cause no distortion or hypertrophy of the host cell but mature gametocytes displace the nucleus and cause slight hypertrophy of the infected cell. Occasionally 2 parasites occur in the same cell. The parasites do not alter the staining reaction of the infected erythrocytes. *Trophozoites.*—The smallest stages are of irregular shape and do not contain visible pigment. As the trophozoites grow they become oval or round with a regular outline, and pigment occurs as fine, round, brown or black granules. The chromatin occupies various positions in the parasite and stains pink; the cytoplasm stains a very light blue and frequently appears to be vacuolate (Figs. 1 and 2). *Schizonts and Segmenters.*—The multinucleate stages are usually round but occasionally they are elongate oval, and they have a delicate appearance due to the small size of the chromatin masses and to the faint blue color of the cytoplasm (Fig. 3). The pigment occupies various positions in the parasite, occurring in schizonts as a loose aggregation of fine granules and in segmenters as a compact clump. Segmenters produce from 6 to 15 round merozoites, the average being 10 (Fig. 4). *Gametocytes.*—The sexual stages are ovoid or irregularly ovoid, depending upon the position in the host cell and the degree to which the host nucleus is displaced. Usually they are ovoid with a regular outline. When the gametocyte occupies a polar position in the host cell the parasite is commonly indented by the host cell nucleus. In other positions it is more regularly round or ovoid (Figs. 5–8). In the macrogametocytes the cytoplasm stains a deep blue, and the nucleus is large and stains faintly. The macrogametocyte nucleus stains a much lighter color than the chromatin in asexual parasites. Mature macrogametocytes contain from 20 to 30 or more fine, round, black pigment granules irregularly distributed through the cytoplasm (Fig. 8). The average size of mature macrogametocytes is 6.9×12.0 μ. In microgametocytes, which always stain faintly, the cytoplasm is almost colorless and the chromatin appears as a diffuse, light pink area (Fig. 7). The pigment is similar to that in the macrogametocytes but consists of fewer granules. Microgametocytes have average dimensions of 5.8×9.2 μ, thus appearing to be more nearly round and smaller than the macrogametocytes.

Host cells: Erythrocytes.
Sporogonous cycle and vectors: Unknown.
Type host: Leiolopisma fuscum.
Type locality: Goodenough Island (New Guinea Area).

UNIDENTIFIED HAEMOSPORIDIA

The blood smears from approximately half of the lizards contained various stages of an unidentified parasite that reproduced by schizogony. The parasites, which in some lizards were relatively numerous, usually occurred adjacent to the nucleus of a ruptured cell. Many of these cells were not identifiable, but occasionally host cells were not ruptured and could be identified as thrombocytes and small lymphocytes. The parasites do not contain pigment and no stages are encountered in erythrocytes. They appear in the form of schizonts, segmenters and uninucleate bodies. The schizonts are usually round or ovoid, have a light blue cytoplasm, and contain as many as 19 discrete, pink masses of chromatin. The schizonts are remarkably similar to the exoerythrocytic schizonts of *P. mexicanum* (Thompson and Huff, 1944) occurring in similar cells. Segmenters occur in small numbers and usually produce from 15 to 20 merozoites. The uninucleate forms are irregularly ovoid with a maximum size of approximately 4.0×8.0 μ and consist of two types. In one group the cytoplasm stains deep blue and contains several small, purple, volutin-like granules, and the chromatin appears as a conspicuous, discrete, reddish-purple area. In the other group of uninucleate organisms the cytoplasm stains very light blue and the chromatin is diffuse and stains light pink. The staining reaction and appearance of the two types of uninucleate bodies resemble the macrogametocytes and microgametocytes of certain species of *Plasmodium,* but these forms differ from the gametocytes of *Plasmodium* in that they lack pigment and occur in cells other than erythrocytes.

These parasites are not constantly associated in their occurrence with any other species of parasite and do not appear to be part of the life cycle of the various organ-

isms with which they are occassionally associated. Possibly this haemosporidian represents a new genus of saurian parasites. We are not aware of any other parasite with similar characteristics; however, we believe a final decision regarding the identity of these organisms should be withheld until additional material has been studied.

HAEMOGREGARINES

Most of the blood smears contained large numbers of macrogametocytes and microgametocytes of *Haemogregarina* which usually occurred in erythrocytes and less frequently in immature red cells. These forms are longer than the host cells and are in the form of a compressed "U." They cause much hypertrophy of the infected cell and displace the nucleus or occasionally split the nucleus longitudinally. They are similar to the gametocytes of *Haemogregarina stepanowi* (Danilewsky, 1885). Occasionally the parasites were extracellular, which may have resulted from the rupturing of the host cells during the preparation of the smear. The free forms are elongate with one end rounded and the other tapering and measured approximately 2.9×28.8 μ. Two gametocytes occasionally occur within the same cell.

In several smears that did not contain *Haemogregarina* the erythrocytes were infected by haemogregarines that were smaller and distinctly unlike the gametocytes of *Haemogregarina*. These forms are elongate with average dimensions of 3.9×13.9 μ. The nucleus stains bright red and the cytoplasm is almost colorless. They resemble the sporozoites of certain Haemococcidia.

TRYPANOSOMA

A majority of the smears contained small numbers of trypanosomes. The true shape and size of these organisms is difficult to determine because they are usually distorted during the preparation of the smears. Most of them are of the large, broad, leaf-like form, with a short flagellum and an undulating membrane that appears to be within the organism. These forms have dimensions ranging from 24 to 35 μ and resemble the larger stages of *T. loricatum* in Congo frogs (Dutton, Todd and Tobey, 1907). In some of the smears a more trypanoform shape was observed with the undulating membrane extending along the side and such forms resembled *T. mabuiae* of the lizard, *Mabuia quinquetaeniata* (Wenyon, 1909). The kinetoplast of the various trypanosomes is close to the nucleus and on the posterior side of it. Definite myonemes are not visible in these trypanosomes.

MICROFILARIAE

Small numbers of these organisms were encountered in several lizards. They do not have a sheath and the average dimensions of several fixed and stained specimens is 4.5×90.5 μ.

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THE PREPARATION AND DEMONSTRATION OF AN ANTISERUM FOR *TRICHINELLA SPIRALIS*[1]

ROBERT P. DORIN

This paper introduces to the field of parasitology the method first described by Hektoen and Welker (1933) of the fixing of the antigen on aluminum cream preparatory to injection for the preparation of antisera. This method has been successfully employed by investigators in the field of organ-antisera, notably Spinka and Weichselbaum (1938), Mann and Welker (1940), Cohen and Freda (1940) and others.

While the investigations described in this paper deal with the larvae of the nematode *Trichinella spiralis,* the problem possesses the broader and more important significance of being applicable to other parasites and to other stages in the life cycle of parasites.

The use of the aluminum cream for the preparation of antisera possesses the following advantages: (a) one injection of antigen suffices, (b) the rabbit produces antibodies for long periods of time, and (c) the material for injection need not be sterile.

MATERIAL AND METHODS

Trichina larvae, free of all host proteins and breakdown products of protein, were adsorbed onto aluminum cream prepared as described by Welker and Tracy (1915), and injected into rabbits according to the method of Hektoen and Welker (1933). Variations in the method of preparing the antigen resulted in different antiserum titer values.

The antigen used to determine the titer of the antisera was a commercial preparation (Eli Lilly and Company) used for skin testing in suspected trichinosis. Since it is prepared by an extraction of the dried and powdered larvae with Coca's solution, it was necessary to bring the pH of the preparation to neutrality by the addition of a few drops of dilute acetic acid. The nitrogen content of the antigen, after neutralization, was determined by means of the Koch-McMeekin micro-Kjeldahl method to be 5.8 mg per cent. It was further determined that this antigen failed to give a positive precipitin reaction when tested with an antiserum prepared to rat tissue by the method of Spinka and Weichselbaum (1938). The rat tissue antiserum reacted positively, when tested with rat tissue autolysates prepared in the manner described by Mann and Welker (1940).

The trichina larvae were isolated from rat tissue by digestion with a solution of 1.5% pepsin and 0.5% HCl. Care was taken to exclude extraneous material by washing the larvae with physiological saline until a negative Biuret test was obtained. Failure to comply with these two essentials invariably results in death of the animal, possibly due to peptone shock.

The antisera to *T. spiralis* were prepared with slight modifications by the method of Spinka and Weichselbaum (1938).

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In the experiments, 400,000 trichina larvae were injected per rabbit. It was determined that suspending the 400,000 larvae in 80 cc saline and adding 120 cc of aluminum cream resulted, after centrifugation, in the desired amount of material for injection per rabbit.

The number of larvae was determined by removing a precipitin pipette full of the well-stirred suspension of larvae and emptying this pipette by placing drops on a large slide. The average number of larvae per 100 drops is then determined under low power of the microscope, multiplied by the number of drops per cc for the particular pipette used and this product multiplied by the number of cc of the suspension. While this method of counting leaves much to be desired, in the hands of an experienced individual, the results are surprisingly constant and reproducible.

Blood was drawn by means of heart puncture two weeks after injection of the antigen. The specimens were allowed to stand at room temperature for two hours and then refrigerated overnight. The blood was then centrifuged and the serum removed. Determination of titer was made with the serum before preservative was added. The values obtained represent the most dilute preparation of the commercial antigen which gives a positive reaction with the antiserum using the micro-precipitin technic.

All antisera failed to react when tested with rat-tissue autolysates.

VARIATION IN PREPARATION OF ANTIGEN

Antigen A was prepared by killing the trichina larvae by placing them, suspended in saline, in the freezing compartment of a refrigerator for two weeks.

Antigen B was prepared by incubating the larvae at 37° C for one week. To accomplish this the larvae, suspended in saline, were placed in a volumetric flask the neck of which was filled with toluene. It may be noted that when viewed under the microscope, the larvae did not appear necrotic.

Antigen C was prepared under conditions similar to those in preparing Antigen B, except that incubation was carried on for 18 days. Even after this time the larvae did not appear necrotic. With both antigen B and antigen C, after removal of the toluene by suction, aluminum cream was added to both the larvae and the saline in which they had incubated.

Antigen D was prepared by a slight modification of the method of fixing the antigen. In this experiment the aluminum cream was added to the larvae and the mixture ground in a ball mill for 36 hours. After grinding for this length of time, the larvae appeared to be in so fine a state as to be indistinguishable when viewed under the microscope. The material was then washed out of the ball mill and off the stones and centrifuged. The centrifuged material was then injected just as in the preceding experiments.

Antigen E was prepared in a manner similar to the method of preparation of Antigen D with the exception that grinding took place for 5 hours. After a 5-hour grinding the larvae had been broken into fractions 1/10 the size of the original larvae.

Injection of these antigens yielded antisera with the following titers:

Antigen	*Titer*
A	1:4,000
B	1:5,000
C	1:5,000
D	1:6,000
E	1:11,000

The titers obtained by the aluminum cream method were compared with those obtained from rabbits which had been orally infected with near lethal doses of larvae. The titers of these latter animals ranged up to 1 : 8,000 depending upon the length of time since infestation.

ABILITY OF THE ACTIVELY IMMUNIZED ANIMALS TO RESIST INFECTION

Three rabbits, which had been previously injected with trichina larvae fixed on aluminum cream to determine which preparation of the antigen results in the highest titer antiserum, were used as the actively immunized animals. These three, along with five normal control animals, were each given orally 20,000 larvae suspended in 10 cc of saline. The material was washed down with 4 cc of fresh water. Of these animals, two of the normal controls died, one on the 10th day, and the second one the 14th day following administration of the larvae. One of the actively immunized animals died on the 32nd day as a result of being bitten by a cage mate.

Beginning on the 43rd day, the animals were killed and equal weights of respective tissues were removed, digested, and the content of trichina larvae determined.

The results show an almost complete immunity of the actively immunized animals to infection (Table 1).

TABLE 1.—*Infestation of tissues of immune and non-immune rabbits*

Rabbit No.	Titer of serum to Trichina	Tongue (215 gm)	Masseter (8 gm)	Diaphragm (7 gm)	Foreleg[1] (52 gm)	Foreleg[2] (52 gm)
403 Control	0	7,800	17,300	16,400	30,500	30,700
470 Control	0	12,000	23,700	32,700	45,000	47,500
501 Control	0	9,600	16,900	25,000	39,000	33,200
494 Immune	1 : 5000	90	120	150	200	800
512 Immune	1 : 6000	0	0	0	0	10

1 and 2 on forelegs in above table refer to different forelegs.

PROPHYLACTIC VALUE OF THE ANTISERUM

Rats of approximately the same weight were selected. Six were given a sublethal dose of 4500 larvae by means of a soft rubber catheter with a mild ether anesthesia. Three of these animals were injected with 9 cc of the immune serum intraperitoneally, in divided doses of 3 cc each, on the day preceding, on the day of, and on the day following administration of the larvae. Similarly, the three other infected animals were given normal rabbit serum. As further controls, two noninfected animals were injected with the same amounts of normal serum on the same three days as the other animals.

Eleven days later the following observations were made: (a) Those animals which had been infected with sublethal doses and had been given immune serum were clean looking, sleek, active, responsive animals. To all appearances they were perfectly normal. (b) Those animals which had been infected with the sublethal dose of larvae and injected with normal rabbit serum possessed bloody-red noses and ruffled fur. They were unresponsive to mechanical stimuli and appeared moribund. (c) The normal serum controls without larvae were completely normal in all respects.

CONCLUSIONS

1. A high titer antiserum to the larvae of *Trichinella spiralis* has been successfully prepared by the aluminum cream method of Hektoen and Welker (1933).

2. The antiserum is specific for the trichina larvae and not for any of the host proteins.

3. This work confirms observations by others that: (a) actively immunized animals can resist infection, (b) immune serum acts as a prophylactic agent to infection with *Trichinella spiralis*.

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NOTES ON THE CHINESE SPECIES OF THE GENUS *PHLEBOTOMUS*. PART VI. SANDFLIES IN CHUNGKING, SZECHUAN, WITH DESCRIPTION OF A NEW SPECIES, *PHLEBOTOMUS KOLOSHANENSIS*

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Little is known of the sandfly fauna in Szechuan Province. Although Hou (1943) reported having found sandflies in the western part of this province, he failed to identify them. In 1943 a systematic survey of sandflies from the beginning of April to the end of October was carried out by the writers in the vicinity of Koloshan, Chungking. The insects began to appear in the latter part of April and disappeared early in October. During the period under survey, recumbent-haired sandflies totalling 875 in number were collected. Of them, 607 were identified to be *Phlebotomus barraudi* while the rest belong to a new species. The morphology of this new species is very characteristic and not identical with that of any of the known species recorded in literature. We propose, therefore, to name it *Phlebotomus koloshanensis* after the place where it was first found.

Phlebotomus koloshanensis n. sp.

A total of 124 males and 144 females of this new species were collected and examined. They are comparatively small insects and dark grey in color. The hairs on the dorsum of the abdominal segments from II to VI are markedly recumbent. Twelve males and ten females were subjected to measurement, the averages of which are given in the description.

1. *Male*

The *total length* of the body is 2.52 mm. This length is about 0.78 times that of the hind leg and 1.29 times that of the wing.

The *pharynx* (Plate I, Fig. 4) is not dilated posteriorly. It measures 0.162 mm in length which is about 3.8 times its greatest breadth (0.043 mm). The pharyngeal armature consists of five to six rows of separate transverse ridges with sharp serrations at their posterior margins. In the center of the armature, a number of separate teeth are present. Several folds of the pharyngeal plate are seen anterior to the armature.

The *buccal cavity* (Plate I, Fig. 3) has a small mushroom-like pigmented area with a broad stalk and a conical cap. The distal half of the cap protrudes into the buccal cavity. The buccal armature consists of four to five rows of small teeth arranged in groups. The number of teeth in each group varies from one to six.

The *antenna* has a formula of 1 over III–XV. It measures 1.70 mm in length which is about 5.5 times that of segment III (0.317 mm) and 5 times the combined length of segments XII–XVI (0.340 mm).

The *palp* has a formula of 1, 2, 3, 4, 5. The lengths of the segments are 0.060, 0.119, 0.124, 0.169 and 0.318 mm, respectively. The combined length of segments 1 and 2 is about 1.4 times that of segment 3. The total length of the palp is about one-half that of the antenna. The Newstead's spines are situated at the basal half of segment 3 and are about 15 to 20 in number.

The *wing* (Plate I, Fig. 1) is lanceolate. Its length of 1.960 mm is about 3.8 times its greatest breadth (0.516 mm). The measurements of the important wing veins as well as their relative ratios are as follows:

α, 0.299 mm; β, 0.385 mm; γ, 0.365 mm;
δ, 0.136 mm; ε, 0.461 mm; and θ, 0.973 mm.
α/β, 0.78; β/γ, 1.05; α/γ, 0.82;
δ/α, 0.45; α/ε, 0.65; θ/ε, 2.11;
β/ε, 0.84; $\alpha+\beta/\theta$, 0.70; and wing length/θ, 2.01.

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The *hind leg* has a length of 3.244 mm, which is about 4.3 times that of its femur (0.759 mm) and 2.8 times that of its tibia (1.157 mm). The first tarsal segment (0.559 mm) measures about one-sixth the length of the leg.

The *male genitalia* (Plate I, Fig. 5). The distal segment of the superior clasper carries 4 curved macrochaetae which have a length of 0.110 mm. Two of these are apical in position while the other two arise from a sub-apical tubercle. A non-deciduous spine is situated about the middle of the segment. The proximal segment measures 0.287 mm in length and is about 1.7 times the length of the distal segment (0.167 mm). The inferior clasper (0.256 mm) is slightly shorter than the proximal segment of the superior clasper. The submedian lamella (0.141 mm) is about one-half the length of the inferior clasper. The intermediate appendage is simple with a spatulate end. The intromittent organ is bifurcated at its tip. The pompetta lies in the abdomen just opposite the junction of the fifth and sixth abdominal segments.

2. *Female*

The *total length* of the body is 2.70 mm. This length is about 0.8 times that of the hind leg and 1.23 times that of the wing.

The *pharynx* (Plate I, Fig. 7) is not much dilated posteriorly. It measures 0.174 mm in length which is about 3.2 times its greatest breadth (0.054 mm). The pharyngeal armature consists of seven to eight rows of separate transverse ridges, each of which is serrated at its posterior margin. A number of separate teeth is present on the posterior part of the armature. Several folds of the pharyngeal plate are seen anterior to the armature.

The *buccal cavity* (Plate I, Fig. 6) has a mushroom-like pigmented area with a short stalk and a large conical cap protruding entirely into the buccal cavity. The buccal armature is in form of a transverse row of continuous teeth, of which four small ones are closely arranged in the center and five to six larger ones are distantly situated on each side. There are five or six separate teeth near the roof of the buccal cavity. A number of small teeth grouped in irregular patches are visible posterior to the armature.

The *antenna* has a formula of 2 over III–XV. It measures 1.525 mm in length which is about 5.4 times that of segment III (0.279 mm) and 4.5 times the combined length of segments XII–XVI (0.339 mm).

The *palp* has a formula of 1, 2, 3, 4, 5. The lengths of the segments are 0.055, 0.095, 0.141, 0.171 and 0.315 mm, respectively. The combined length of segments 1 and 2 is about equal to that of segment 3. The total length of the palp is about one-half that of the antenna. The Newstead's spines are situated at the basal half of segment 3 and are about 40 to 50 in number.

The *wing* (Plate I, Fig. 2) is lanceolate. It measures 2.188 mm in length which is about 3.7 times its greatest breadth (0.596 mm). The measurements of the important wing veins as well as their relative ratios are as follows:

α, 0.409 mm; β, 0.427 mm; γ, 0.373 mm;
δ, 0.221 mm; ε, 0.545 mm; and θ, 1.087 mm.
α/β, 0.96; β/γ, 1.14; α/γ, 1.09;
δ/α, 0.54; α/ε, 0.75; θ/ε, 1.99;
β/ε, 0.78; $\alpha+\beta/\theta$, 0.77; and wing length/θ, 2.01.

The *hind leg* has a length of 3.375 mm, which is about 3.8 times that of its femur (0.883 mm) and 2.9 times that of its tibia (1.165 mm). The first tarsal segment (0.575) measures about one-sixth the length of the leg.

The *spermatheca* (Plate I, Fig. 8) measures 0.067 mm in length and 0.021 mm in breadth. It is cylindrical in shape with a trumpet-like opening. Enclosed in the trumpet, there is a small distal segment from which a cluster of filaments arises. The spermatheca is bent backward abruptly from a short and broad spermathecal duct. On both the duct and spermatheca, faint striations are visible.

DIFFERENTIAL DIAGNOSIS

The presence of numerous recumbent hairs on the dorsum of the abdominal segments II to VI indicates that this newly found species belongs to the recumbent-haired division. The specific morphological characters of the buccal armature as described above together with the presence of the mushroom-like pigmented area which protrudes either partly or completely into the buccal cavity differentiates this new species from all other recumbent-haired ones. The morphology of the spermatheca and the arrangement of the macrochaetae on the superior clasper are also of diagnostic importance.

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Explanation of Plate I

Camera lucida drawings of the important structures of *P. koloshanensis* n. sp.

Fig. 1. Wing, male.
Fig. 2. Wing, female.
Fig. 3. Buccal cavity, male.
Fig. 4. Pharynx, male.
Fig. 5. Male genitalia.
Fig. 6. Buccal cavity, female.
Fig. 7. Pharynx, female.
Fig. 8. Spermathecae.

PLATE I

1

2

0.5 mm

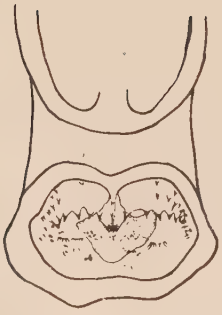
3

4

0.05 mm

5

0.3 mm



6

7

.002 mm

8

RESEARCH NOTES

A NOTE ON THE NOISE-MAKING ABILITY OF THE TSETSE-FLY, *GLOSSINA PALPALIS* ROB.-DESV. (DIPTERA, GLOSSINIDAE), WHILE IN FLIGHT

There seems to be some discussion in the literature regarding the noise-making ability of the medically important species of tsetse-flies. The name "tsetse" has generally been considered to be an onomatopoetic designation given by South African natives to the buzzing sound of *Glossina morsitans* when in flight. Austen (1903, Monograph of the Tsetse-Flies, 1) says, "There is no doubt that the word Tsetse (Tsitsi) is onomatopoetic and that since it has been employed from the beginning to designate *Glossina,* it owes its origin to the peculiar buzzing sound made by the fly on the wing or when commencing to suck blood." Hegh (1929, Les tsé-tsés, Tome I, Annex to 1st Chapter) also maintains that ". . . the word 'tsé-tsé,' in its English pronunciation 'tsi-tsi,' is a repetition of the word 'tsi,' onomatopoetically imitating the characteristic noise which the flies of the genus *Glossina* make in flying."

On the other hand, Bequaert (1930, Medical and Economic Entomology, 990) in the report of the Harvard-African expedition to Liberia and the Belgian Congo, states, "In my experience, *Glossina palpalis,* at any rate, has a perfectly noiseless flight and it alights or flies off without giving much warning to its victim. So far as I remember, the other species of *Glossina,* including *G. morsitans,* make no appreciable noise *in flight.*" He then goes on to say that "All tsetse-flies, however, make at times a high, shrill, singing noise, *when resting,* before or after feeding."

The central point in question appears to be whether these flies produce a noise *while in flight.* Incidental observations were made on this question by the authors during the course of collecting trips for *Glossina palpalis* Rob.-Desv. in the Dakar region of French West Africa. Collecting trips for this fly, which extended through most of 1944 and the early part of 1945, habituated us to its characteristics.

Most of the trips to the "tsetse belts" began in the early morning and extended through the early afternoon. It was noted that *G. palpalis* had a rather noiseless flight during the early morning hours when the air was relatively cool, especially so within the thick groves which constituted the haunts of these flies. As a matter of fact, this relatively noiseless flight in the morning hours was of some concern to us during our first collecting trips when we had not yet become accustomed to the fly's habits. At that time one of us was bitten by *G. palpalis* since, in the words of Bequaert, it gave no "warning to its victim." Later on we established the practice of walking single file through the groves so that a sharp lookout for attacking flies could be maintained.

We quickly observed, however, that *G. palpalis* produced a very distinct and characteristic buzzing noise as the air temperature within the groves moved upwards. At first we were somewhat confused by the sounds of other flies, mainly muscids and tabanids. However, we very quickly became cognizant of the peculiar buzzing noise of *G. palpalis* while in flight and this sound turned out to be a very excellent warning. The intensity of the noise was observed to be directly correlated with temperature increase and it was at its height during the noonday and afternoon heat. During these portions of the day, it was the sound of the fly, while in flight, that constituted the first warning that tsetses were near and thus contributed to the success of our collecting with nets. This was in direct contrast to early morning collecting when its success was more or less dependent upon first spotting the fly as it alighted upon our dark clothing.

Our experience with *G. palpalis* at Dakar seems to be in direct contrast with the observations of Bequaert on this species in Liberia and the Belgian Congo. It may be possible that the species exhibits physiological differences as between the two regions. It is certainly clear, at any rate, that *G. palpalis* at Dakar produces a characteristic buzzing sound *while in flight* and which appears to be directly dependent upon the atmospheric temperature within the tsetse groves.—2D LT. LEO KARTMAN, *Sanitary Corps, Army of the United States;* TECHNICAL SERGEANT EDWARD J. CAMPAU; TECHNICIAN 4TH GRADE ELDON H. NEWCOMB; *and* TECHNICIAN 5TH GRADE FREDERICK D. MORRISON, *Medical Department, Army of the United States.*

A NOTE ON ANOPHELINE VECTORS OF *WUCHERERIA BANCROFTI* IN WEST AFRICA

Annett, Dutton, and Elliott (1901, Liv. Sch. Trop. Med., Mem. IV, 69–72) found in Nigeria that 5.7% of 281 *Anopheles gambiae* were infected with *W. bancrofti* larvae. Taylor (1930, Ann. Trop. Med. and Parasitol. **24:** 425–435) working in Northern Nigeria, showed that of 1936 *A.*

gambiae dissected, 1.65% had mature filaria larvae, 6.97% had immature larvae, while the total rate of infection was 8.62%. Of 1260 *Anopheles funestus* dissected, 0.87% had mature larvae, 3.17% had immature larvae, and the total infection rate was 4.05%. He concluded that *A. gambiae* and *A. funestus* were the primary vectors of *W. bancrofti* in that region and that *A. gambiae* is a little more than twice as heavily infected as *A. funestus*. Hicks (1932, Ann. Trop. Med. and Parasitol. **26**: 407–422) found that under laboratory conditions both *A. gambiae* and *A. funestus* allowed complete development of *W. bancrofti* larvae. He also demonstrated that mature larvae dissected from experimental *A. gambiae* and *A. funestus* were indistinguishable from larvae found in wild specimens of these two anopheline species.

During the early part of 1944 the author made some incidental observations on this problem at Dakar, Senegal, French West Africa. More or less sporadic dissection of *A. gambiae* and *A. funestus* from four native villages within a radius of 12 miles eastwards from Dakar confirmed the results of other workers that these two anophelines are excellent hosts of *W. bancrofti* under natural conditions. Negroes suffering with elephantiasis were seen in some of these villages.

TABLE 1.—W. bancrofti *in anopheline mosquitoes from four native villages*

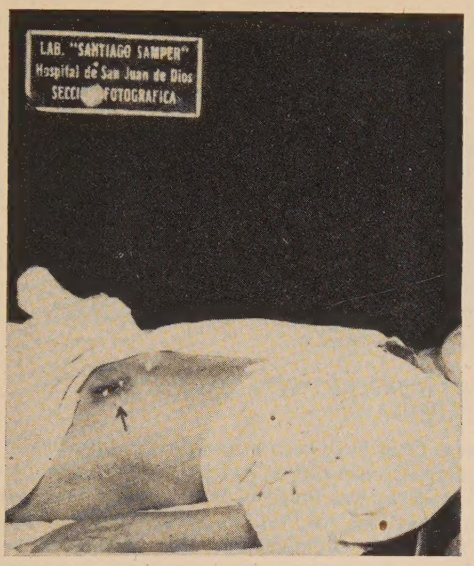
Date (1944)	Village	Host	Number Dissected	No. filaria found in					Number Infected	Per cent Infection
				Gut	Abdomen	Thorax	Head	Proboscis		
1–15 to 5–17	A	*A. gambiae*	79	15	0	58	0	1	39	49.3
		A. funestus	351	24	0	63	0	0	65	18.5
1–21 to 5–17	B	*A. gambiae*	23	3	0	2	0	0	3	13.0
		A. funestus	82	0	0	2	0	1	2	2.4
1–17 to 5–15	C	*A. gambiae*	72	61	0	44	9	0	39	54.1
		A. funestus	223	26	0	18	0	0	41	18.3
5–30 to 6–16	D	*A. gambiae*	200	19	3	44	4	0	56	28.0
		A. funestus	90	2	3	7	1	0	9	10.0
Total		*A. gambiae*	374	98	3	148	13	1	137	36.6
		A. funestus	746	52	3	90	1	1	117	15.6

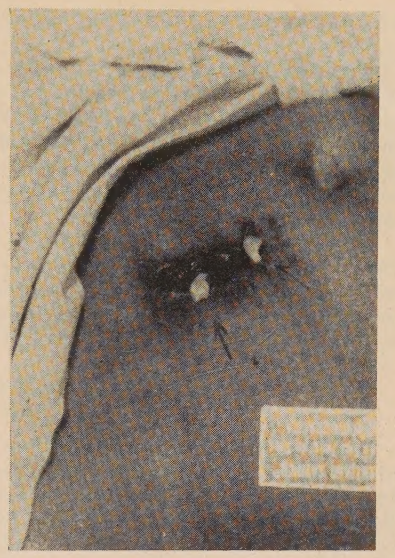
Table 1 gives data on incidence of infection of *A. gambiae* and *A. funestus* with *bancrofti* larvae.

The habits of *A. gambiae* and *A. funestus* in this region are well known. In routine checking of native villages during malaria surveys it was found that these two species were predominant throughout the year. They far outnumbered all other mosquitoes including *Culex fatigans* (= *quinquefasciatus*). These extreme domestic habits suggest that they are the primary vectors of Bancroft's filaria in the Dakar region. *A. gambiae* was seen to be a bit over twice as much infected with filaria larvae as *A. funestus* and it has been suggested that, although *funestus* is an efficient vector, it has a lower infection rate since it is a smaller species and takes less blood than *gambiae*.—2D LT. LEO KARTMAN, *Sanitary Corps, Army of the United States.*

EXAMINATION OF TUBE-DWELLING POLYCHAETE ANNELIDS FOR LARVAL TREMATODE INFECTIONS

The reports of Linton (1915, Biol. Bull. **28**: 115–118), Stunkard (1929, Anat. Rec. **44**: 264) and Martin (1944, Trans. Am. Micr. Soc. **63**: 237–243) that larval trematodes may develop in the polychaete annelid *Hydroides dianthus*, emphasizes the need for examining other invertebrates than molluscs as possible intermediate hosts for these flatworms. Accordingly, during the summer of 1945, fifteen species of tube-dwelling polychaetes were examined, of which only 12 of 1500 *Hydroides* were found positive (and these with *Cercaria loossi*). Those species found negative were: 75 *Amphitrite ornata*, 25 *Arenicola cristata*, 25 *Chaetopterus variopedatus*, 62 *Cistenides gouldii*, 300 *Clymenella torquata*, 40 *Diopatra cuprea*, 35 *Dodecaceria coralii*, 20 *Maldane elongata*, 12 *Parasabella micropthalmia*, 72 *Pista palmata*, 15 *Praxillella zonalis*, 30 *Pseudopotamilla oculifera*, 43 *Sabellaria vulgaris*, and 2000 *Spirorbis spirorbis*. Further examinations of these and other invertebrate species is being continued.—JOHN S. RANKIN, JR., *Zoology Department, University of Connecticut and the Marine Biological Laboratory, Woods Hole.*





EXPULSION OF TAPEWORM PROGLOTTIDS FROM ARTIFICIAL ANUS*

A woman 23 years of age with an artificial anus expelled regularly through it the ripe proglottids of *Taenia saginata.* The largest number that escaped in a single day was 40. Figures 1 and 2 show the proglottids escaping.—Dr. A. Bonilla-Naar. Hospital de San Juan de Dios. Bogotá-Colombia, S. A.

NEW HOST RECORDS FOR THE TROMBICULID MITE, *ACARISCUS MASONI* EWING

In the notes accompanying the original description of *Acariscus masoni* Ewing (1943, Proc. Ent. Soc. Wash. 45(3) : 61) there is a host list of 10 species in which the only non-mammalian species is a "black snake (species?)." In August 1945 while observing investigations on acaricides at the laboratory of the Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, U.S.D.A., at Orlando, Florida three previously unrecorded hosts of this species were collected. A southern soft-shelled turtle, *Amyda ferox* (Schneider) (U. S. National Museum Collection, No. 121568) was found to have 253 attached partially engorged larvae. Of these 114 were attached to the carapace and were apparently as well engorged as those on other parts of the body. The other specimens were found on the legs, neck, eyelids, and in the external nares. On a six-lined lizard, or race-runner, *Cnemidophorus sexlineatus* (L) (U. S. National Museum Collection, No. 121569), about twenty partially engorged larvae were found. These were in the external nares and on the hind legs. A single partially engorged larva was found on the right hind leg of an immature tree frog, probably *Hyla squirrela* Latreille (U. S. National Museum Collection, No. 121573–74). These hosts were collected near Orlando, in an old, partially dry, lake bottom in which the larvae of *Acariscus masoni* Ewing were extremely abundant. Larval specimens collected from this location, both from boot samples in the field and from the above-mentioned hosts, have been deposited in the collections of the U. S. National Museum, Washington, D. C., and the Rocky Mountain Laboratory, U. S. Public Health Service, Hamilton, Montana.—D. S. Farner and Elwood A. Seaman, *Lieutenant H(S) USNR and PhM 2/c USNR, respectively, U.S.A. Typhus Commission.*

NEW HOST RECORDS FOR *MICROPHALLUS OVATUS* OSBORN, 1919

Since February 27, 1943, 127 turtles, representing 7 species, have been examined for parasites. All of the turtles were collected in Ohio, and most of them from the central part of the State. Of this number, 4 turtles were infected with *Microphallus ovatus.* The hosts with the number of parasites in each were as follows: 2 specimens of *Chrysemys bellii marginata,* 62 and 500 plus; one specimen of *Chelydra serpentina,* 500 plus; one specimen of *Graptemys geographica,* 2.

One specimen of *C. b. marginata,* collected on May 30, 1944, in Marion County, in addition to the adult flukes, had many metacercariae in the intestine which showed much variation in size

* *Faust's opinion:* "It is most interesting and in so far as I know unique." . . .

and state of maturity. The other host of this species, collected September 21, 1944, from Union County, had many flukes *in coitu*.

The stained flukes were very typical of *M. ovatus* and did not resemble *M. opacus* (Ward, 1894). All specimens examined showed two intestinal caeca of equal length, in contrast to the condition of only one as described by Van Cleave and Mueller (1934, Roosevelt Wild Life Annals 7: 227–229). It is of interest that the infected turtles were collected only from streams. This may be due to the fact that many of the ponds, from which turtles were collected, had few or no crayfish. It is very likely, however, that this distribution is also dependent upon a snail host not found in the ponds.

Previous records of *M. ovatus* are all from fish; they are small-mouthed black bass (*Micropterus dolomieu*), Bullhead (*Ameiurus* sp.) rock bass (*Ambloplites rupestris*), and pickerel (*Esox niger*). In no instance were many parasites found in a fish. It is obvious that the turtles mentioned above are satisfactory hosts.—Robert Rausch, *Dept. Zoology and Entomology, Ohio Cooperative Wildlife Research Unit, Ohio State University.*

NEW RECORDS OF *MACRACANTHORHYNCHUS HIRUDINACEUS* IN SCIURIDAE

A number of squirrels collected from central Ohio have been examined for parasites in the past few years. Among these were the fox squirrel (*Sciurus niger rufiventer*) and the chipmunk (*Tamias striatus*). *Macracanthorhynchus hirudinaceus* was found in 2 specimens of the former, collected on September 23, 1943, near Radnor, Delaware Co., Ohio. In one squirrel, 3 adult specimens were found in the small intestine, and one in the abdominal cavity, attached to the abdominal wall. Since a few hours elapsed before the squirrels were autopsied, it is possible that this individual migrated from the intestine after the death of the host. There was no evidence of its having done so, however, since the intestine was intact. A second squirrel, collected from the same woodlot, contained one specimen of this parasite in the small intestine. The squirrels, both males, were in apparently normal physical condition, and gave no gross clinical evidence of the presence of the parasites. No other parasites were present in either animal.

A specimen of *T. striatus* was collected on June 18, 1944, about 10 miles north of the above locality, in Marion Co., Ohio. An immature specimen of *M. hirudinaceus* was found attached to the mesentery. This abnormal location might be due to the presence of the parasite in an unnatural host.

These specimens, so far as I have been able to determine, represent the first recorded from members of the Sciuridae. Katz (Abstract of thesis, *The Parasites of Ohio Squirrels,* in Release No. 131, Ohio Cooperative Wildlife Research Unit) does not record this parasite. He examined 88 squirrels, 16 of which were *S. niger rufiventer*.

That the fox squirrel seems to be a satisfactory host for this parasite is of considerable interest, since it could act as a reservoir host, and keep an area infected, even though swine were not present for a number of years. Swine were present in the woodlot from which these squirrels were collected.

I am indebted to Dr. H. J. Van Cleave for aid in the identifications, and to him and Dr. E. W. Price for information concerning previous host records.—Robert Rausch, *Dept. of Bacteriology and Public Health, Michigan State College, E. Lansing, Michigan.*